An investigation of response decrement

in jumping spiders

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Table of contents

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Thesis abstract

Paying attention to a stimulus is costly in terms of cognitive resources. Given the high number of stimuli (and the low salience of many of them), animals filter out a large amount of irrelevant information, but exactly how this is done is not entirely understood. The decision as to which stimuli to ignore is risky, as mistakes can be fatal. Habituation describes a decrease in response to repeated biologically irrelevant stimuli over time. I investigated characteristics of the response decrement in *Trite planiceps*, a New Zealand jumping spider which is an active visual hunter, by measuring their response decay to repetitive visual stimuli. Spiders were tethered in front of two stimulus presentation monitors and were given a polystyrene ball to hold. Movement of this ball indicates an attempt to turn towards a visual stimulus presented to a specific pair of laterally-facing eyes (anterior-lateral eyes). Response decay is easily measured, as moving visual stimuli trigger clear responses, which were recorded as four categorical variables: optomotor (very fast); fast response; general movement; no movement. Visual salience (conspicuousness) of the stimulus, biological salience of the stimulus, and, to a lesser extent, spider hunger all affected the rate of decay (response decrement). I then tested whether the observed response decay was likely regulated in the peripheral nervous system (suggestive of classic sensory habituation) or the central nervous system. In paired tests, I gave spiders a drop of either water or caffeinated water to drink. As caffeine is a central nervous system stimulant, I predicted that there would be more responses over a longer period of time in the caffeine treatment if response decrement was centrally regulated - as proved to be the case. These findings support the hypothesis that response decrement in jumping spiders is centrally regulated.

Chapter 1
Introduction

Arthropod cognition

Historically, there has been a widespread belief that arthropod behaviour was guided primarily by instinct and innate routines and that they were incapable of learning (Alexander et al., 1997; Dukas, 2008). Take, for example, courtship behaviour in *Drosophila*. Males know how to court without exposure to another animal (i.e., this is innate behaviour), and the steps comprising courtship nearly always occur in the same order (Manoli et al., 2006). The series of actions include orienting towards and following the female, tapping her with his forelegs, singing a species-specific courtship song by vibrating one of his wings, licking the genitalia of the female, and curling his abdomen to attempt copulation (Hall, 1994; Greenspan and Ferveur, 2000; Manoli et al., 2006). The innate nature of this behaviour suggests that the underlying neuronal substrates necessary for their execution are genetically determined (Manoli et al., 2006). Baker and colleagues (2001) argued against environment shaping certain behaviours in *Drosophila*, stating that a significant number of behaviours (e.g., fixed action patterns and species-specific innate mating behaviours) are relatively unaffected by the environment and thus appear to be dictated by genes. From these early examples suppositions regarding the cognitive ability of all arthropods were made - suppositions which only now are being scientifically questioned (Alexander et al., 1997; Dukas, 2008).

The idea that arthropods lack cognitive ability was based on two assumptions: that their lifespan is too short for learning to be beneficial (Alexander et al., 1997) and that arthropod brains are too small to process and produce complex behaviour (Dukas, 2008). Observational data supported the instinct-driven notion at the time. However, many arthropods are extremely long-lived; with some species of spiders, for example, living thirty years or more (Danks, 1992; Costa and Pérez-Miles, 2002). Only recently have we been able to recognise innate behaviour as separate from cognitive ability (Alexander et al., 1997; Dukas, 2008). Indeed, it is almost certain that learning is a requirement for coping with changing environments (Alcock, 2005), so even if the lifespan of a given animal is relatively short (e.g., a year), the potential opportunity to employ knowledge learned throughout this lifespan could be selected for through increased reproductive success.

Complex cognitive abilities, such as concept formation (Chernikova et al., 2008), rule learning (Smith et al., 2011), numeracy (Hauser, 2000) and categorisation (Smith et al., 2016) are often attributed to vertebrates (Pepperberg, 2009). However, these abilities have been more

recently demonstrated in insects and other arthropods, including spiders (Jackson and Wilcox, 1993; Wilcox et al., 1996; Tarsitano and Jackson, 1997; Harland and Jackson, 2004; Jackson and Cross, 2011), praying mantises (Kral and Prete, 2004) and bees (Dacke and Srinivasan, 2008; Menzel, 2012; Loukola et al., 2017). For example, Loukola and colleagues (2017) explored bumblebee behavioural flexibility in a task where they were required to transport a ball to a defined location in order to receive a reward. Bees were pre-trained to know the defined location. Untrained bees observed the demonstration of the technique from a live bee or a model bee attached to a thin transparent stick moved by the experimenter. Later, the experimenters moved the apparatus to a bigger platform, and the previously trained bees observed a new demonstration of the technique through a "ghost" (ball moved via magnet) or received no demonstration. Loukola et al., (2017) found that bees that observed the live or model demonstration learned the task more efficiently than the bees that observed the ghost or bees that received no demonstration. They also found that, instead of copying the task in the same way as demonstrators which had moved balls over long distances, the observers solved the task more efficiently by using the ball closest to the target, even when it was a different colour to the one previously observed. This exceptional cognitive flexibility could suggest that if relevant ecological pressures arise, entirely new behaviours could emerge relatively quickly in species whose lifestyle demands advanced learning abilities - whether those species are vertebrate or not (Loukola et al., 2017).

Spiders are also capable of gaining information from experience and adjusting their behaviour in a multitude of tasks, such as anti-predator activity, aggression, social interaction, mate choice (Dukas, 2008; Jakob et al., 2011), as well as intraspecific conflict (Whitehouse, 1997a) and foraging (Jackson and Wilcox, 1993). Whitehouse (1997b) provided an example of experience influencing spider behaviour in contests. Male *Argyrodes antipodianus* were trained to be winners or losers during intraspecific duels. Winners were paired with a smaller combatant during training to give them the experience of winning while losers were paired with larger combatants. She found that when matched with combatants of the same size, the winners were more likely to continue to win, and vice versa for the losers. These results could be because the spiders undergo self-assessment and determine their ability and fighting reserves (Whitehouse, 1997b). The same has also been demonstrated in other families, such as crab spiders (Dodson and Schwaab, 2001) and jumping spiders (Kasumovic et al., 2009).

The abilities demonstrated in the examples above suggest that small brains can produce complex behaviours. Large brains often only show quantitative improvements when compared with

smaller brains (Chittka and Niven, 2009), so the cognitive abilities of arthropods should not be dismissed solely because of their brain size. It is likely that cognitive ability lies on a continuum, with some animals at one end of the spectrum performing predominantly innate behaviour, and others being capable of higher information processing and decision-making (Jackson and Cross, 2011). Where a particular animal lies on this continuum depends on several variables, including life history traits, phylogeny and environmental stochasticity. Although an animal's precise location on the continuum is hard to pinpoint, the ability to learn to overcome challenges in their environment from experience may be a good place to begin (Jackson and Cross, 2011).

Research into the ability of spiders to learn parallels that of other arthropod learning, and there is now a fair amount known about complex cognitive abilities in several spider species. However, fewer studies have looked at basic cognitive attributes, both among insects and spiders (Dukas, 2008; Jakob et al., 2011).

Habituation and response decrement

The most fundamental form of adaptation to experience is habituation (Dong and Clayton, 2009), as exemplified in the diversity of life in which it is found: from the unicellular slime mould *Physarum polycephalum* (Boisseau et al., 2016; Vogel and Dussutour, 2016), to humans (Geer, 1966). Habituation is a form of non-associative learning which is demonstrated as a decreased neural or behavioural response, including complete cessation, to a repeated stimulus (Mazur, 2012). This decline in responses is called a response decrement. An example is when people put on their glasses or clothes. Initially, they can feel the sensation of the weight on their skin, but that sensation quickly diminishes. Habituation enables an animal to distinguish biologically irrelevant background stimuli from biologically relevant stimuli. This is important, as the consequence of making a classification mistake between biologically relevant and non-relevant could be disastrous (Christensen et al., 2008).

Habituation acts like a filter and prevents an organism's nervous system from being flooded with irrelevant information (Klingner et al., 2014). Any natural environment holds a plethora of information, much of which requires sensory processing: prey, predators, potential mates, as well as vibrations, shadows and general surroundings (Prete, 2004). Nervous systems must process sensory information, categorise that information, and, based on that categorisation, make a decision to either flee, ignore, or approach the stimulus. For example, an animal should pay attention to the movement of a potential predator, but not to leaves moving in the wind and the

shadows that they cast, which should be ignored. If an animal were to process every piece of irrelevant information all the time, the nervous system would certainly be overloaded, and arguably this should be more pronounced among animals with smaller nervous systems (Dukas and Clark, 1995; Prete, 2004; Christensen et al., 2008).

Animals are limited in their sensory processing abilities, and there is a fine balance between picking up on a stimulus when needed, and unnecessarily flooding the nervous system with surplus input (Wiley, 1994; Bradbury and Vehrencamp, 1998). Detection of a predator before an attack is critical for an animal's survival if it is detected in time for the potential prey to flee (Yamawaki and Ishibashi, 2014). Because experience with a predator can be lethal, early researchers studying birds suggested that predator recognition did not require learning (Tinbergen, 1948). However, we now know that is not always the case, certainly among vertebrates (Lodish et al., 2000; Schloegl et al., 2007). Many of the cues associated with a predator can be highly variable and change over time, so it is advantageous for animals to modify their predator recognition behaviours through learning and experience (Deecke et al., 2002).

Deecke and colleagues (2002) investigated the response of harbour seals (*Phoca vitulina*) to the underwater calls of killer whales (*Orcinus orca*). They played calls from three different killer whale populations to a seal population. The calls included populations from mammal-eating killer whales, which on occasion made attacks on the targeted seal population (transient orca), unfamiliar calls from fish-eating killer whales 600 km away from the seal population, and calls from familiar fish-eating killer whales that lived in the local area. They found that the seals swam further away from the area when calls of mammal-eating killer whales and unfamiliar fish-eating killer whales were presented, but not when the familiar calls of the local fish-eating population were presented. In other words, the seals selectively modified their "predator image", based on their calls. The authors argue that the difference in responses is due to selective habituation to the calls of harmless killer whales, rather than to associative learning; as the seals had no experience of the distant fish-eating killer whale calls, but still responded strongly (Deecke et al., 2002).

Nervous systems are metabolically costly and are limited in their ability to maintain high-quality information processing for extended periods of time (Dukas and Clark, 1995). With the vast amount of sensory information that must be processed, this is especially likely to be evident in animals with small nervous systems, such as insects and spiders (Chittka and Niven, 2009). Research using invertebrates is, therefore, a very useful platform from which to understand further

how nervous systems facilitate learned behaviour and change due to experience (Martinez and Kesner, 1998). Habituation has been demonstrated in a variety of invertebrates, including fruit flies, *Drosophila melanogaster* (Engel and Wu, 1996), bumblebees, *Bombus impatiens* (Plowright et al., 2006), crayfish, *Procambarus clarkia* (Glantz, 1974; Araki and Nagayama, 2005), cuttlefish, *Sepia officinalis* (Purdy et al., 2006) and squid, *Lolliguncula brevis* (Long et al., 1989). However, habituation has been largely ignored among spider studies, with a single study done on the response decrement of jumping spiders to a repeated visual stimulus (Melrose, 2015).

Attention and salience

Nervous systems sort relevant from irrelevant stimuli within their surroundings. From the human optic nerve alone, the number of incoming stimuli is estimated to be approximately 107-108 bits per second (Itti and Koch, 2001). Instead of attempting to process all this sensory input, attention, like habituation, filters out the relevant information from the irrelevant (Itti and Koch, 2001). Imagine the following experience: you are in a crowded restaurant. Your dinner companion senses a diabetes event coming on, and you go out to get their medication from the car. When you walk back into the restaurant, your friends at another table ask why you just ignored them. They were waving at you, and you looked right at them but did not see them. The vividness of our visual experience leads us to believe that our visual *representations* will include and preserve the same amount of detail as real life (Levin and Simons, 1997; Simons and Chabris, 1999). However, we occasionally fail to notice changes in our surroundings or those around us (Simons and Chabris, 1999).

The attentional filter theory was proposed by Broadbent (1958) and is based on the concept that all sensory information reaches a bottleneck (attention). In other words, a person notices sensory information that appears to be relevant to their task. Simons and Chabris (1999) produced a video of the selective attention task (see: https://www.youtube.com/watch?v=vJG698U2Mvo). In this video, the audience is required to count the number of basketball passes between the six players on screen. Half of the players are in white clothes; the others are in black. The players are constantly moving around as well as passing the ball, so it is a demanding attentional task. At some time during the video, a man in a gorilla suit walks right between the players, stops and bangs his chest facing the camera, and then walks out. At the end, the audience is asked to recall how many passes they counted and if they noticed the gorilla. During this study, 92% of participants who focussed on the players in white did not 'see' the man in the gorilla suit until it was pointed out to

them. Not surprisingly, the people who watched the clip passively, without any task of counting, noticed the gorilla immediately. Interestingly, 67% of the participants focusing on black players noticed the gorilla. This outcome was due to the different information characteristics the participants were focused on; when asked to focus on the players in white clothing, white became the characteristic their attention was focused on, and they filtered out the remaining information (Simons and Chabris, 1999). The bottleneck concept of attention is similar to habituation except attention focuses on relevant stimuli and can move to other stimuli (selective attention), whereas habituation responds initially to a stimulus and, if irrelevant, filters it out (Shinn-Cunningham, 2008).

To manoeuvre successfully in everyday settings animals need to be able to both focus and shift attention as the need arises (Shinn-Cunningham, 2008). Selective attention is how humans and many animals achieve this. An example of selective attention is demonstrated through the 'cocktail party effect'. This describes the phenomenon of focussing one's auditory attention on a particular stimulus while filtering out a range of other stimuli, much in the same way that a partygoer can focus on a single conversation in a noisy room (Bronkhorst, 2000; Shinn-Cunningham, 2008). The cocktail party effect also describes a similar phenomenon whereby an individual may immediately detect words of importance (e.g., their name) originating from unattended stimuli, such as a partygoer in conversation across the room (Wood and Cowan, 1995; Conway et al., 2001). Shapiro and colleagues (1997) found a visual cocktail party effect through target-probe experiments. Participants were required to identify, among fast moving words, a noun or name (target) as well as the presence or absence of a second target (probe), which was their name, another name, or a specified noun. They found that participants were more likely to see the probe when it was their own name, could see the probe some of the time when it was another person's name, but very rarely detected a noun probe (Shapiro et al., 1997). With selective attention acting like a filter, what gets through is an important aspect of vigilance studies.

Salience affects the focus of attention, especially sustained attention (Itti and Koch, 2001; Helton and Warm, 2008). Sustained attention enables the maintenance of vigilance, selective attention, and continuous effort, despite changing conditions (Cohen, 2011). If a given stimulus is not salient, an animal's attention may never be driven to it, which is how camouflaged caterpillars avoid predation from birds (Lichter-Marck et al., 2015). If a stimulus is sufficiently salient, it will 'pop-out' of a visual scene and likely attract attention (Itti and Koch, 2001). Salience is employed in

experiments such as target-probe (Shapiro et al., 1997), priming (Dillman Carpentier, 2011) and selective attention (Chen, 2005) studies. Deak and Warm (2008) investigated salience in sustained attention tasks in humans. Participants watched repetitive presentations of capital letters ‘‘O,” ‘‘D,” and a ‘‘backwards D”; their task was to press a key on a response pad when they spotted the letter “O”. Participants were randomly assigned low signal salience (low contrast to background) or high signal salience (medium contrast to background). The authors found that detection probability was poorer for low salience signals compared to high salience signals and the vigilance decrement was steeper for low salience signals. These results were congruent with previous findings (Warm, 1993; Matthews et al., 2000; Temple et al., 2000). Salience influences what we ‘see’ and is a useful attribute to manipulate in sustained attention studies.

To gain an understanding of how sustained attention is facilitated at the basic level, it would be helpful to investigate a simpler nervous system that is capable of sufficiently complex behaviour. Carrying on from what we know about habituation in invertebrates (Glantz, 1974; Long et al., 1989; Engel and Wu, 1996; Araki and Nagayama, 2005; Plowright et al., 2006; Purdy et al., 2006; Melrose, 2015), I aimed to investigate vigilance decrement in jumping spiders further. Throughout this thesis, I define habituation as a decrement in response to visual stimuli which is due to some form of a decrease in response of the peripheral nervous system (this could be thought of as local control). In contrast, I define response decrement or decay, as a behavioural decrement in response to repetitive visual stimuli, where the mechanism underlying it might come under central nervous system control. If the response decrement is centrally nervous system controlled, this has implications for selective attention and vigilance, which is the action or state of keeping careful watch for possible danger (e.g., air traffic controllers).

Study Animal

The spider family Salticidae, commonly known as jumping spiders, have been subjects in many studies involving complex behaviour because they display cognitive attributes and visual ability comparable to those found in mammals (Land, 1974; Tarsitano and Jackson, 1994; Dukas, 2008). Salticids, having brains the size of poppy seeds, demonstrate the trade-off between complex behaviour and minimal neuronal capacity (Jackson and Harland, 2009; Menda et al., 2014).

The Salticidae is the largest and most diverse spider family, encompassing over 5400 described species in 575 genera (Platnick, 2012). Salticids are found in a plethora of habitat types, from deserts to mountain tops; they are found in every continent on earth, except Antarctica

(Wanless, 1975; Platnick, 2012). Salticids are comparatively small spiders (3-5 mm), with short legs and a broad, square cephalothorax that holds strikingly large eyes. Most salticids are active during the day and prefer bright sunlight and a dry climate (Foelix, 2011). As cursorial hunting spiders, salticids do not use webs to catch prey, but instead use silk to build retreats in sheltered spaces to protect themselves and their eggs from predators and harsh climate. Even a passing observation of salticid behaviour will reveal an apparent inquisitiveness towards visual stimuli; moving objects will almost always catch a salticid's attention (Homann, 1928). Typically, after a quick turn to face the object, the spider will move closer, and in the case of potential prey slowly stalk it and ultimately pounce (Foelix, 2011). It is this behaviour that earned salticids their name (Latin for "to jump"). Hunting their prey requires high visual acuity and advanced visual sensory processes, and this may be a precursor to the high cognitive ability that is an especially apparent trait in this family (Foelix, 2011)

Salticids have a surprisingly small nervous system, containing roughly 500,000 neurons (Mike Land pers. comm. with Ximena Nelson), as opposed to billions of neurons in mammals. Despite this, salticids are renowned for their visually-guided predatory behaviours that are strikingly complex and flexible (Jackson and Wilcox, 1993; Wilcox et al., 1996; Tarsitano and Jackson, 1997; Tarsitano and Andrew, 1999; Harland and Jackson, 2004). Some salticid species go to great lengths to obtain their preferred prey. *Portia fimbriata* hunts web-building spiders, which are often potentially dangerous, and may take long detours to reach an advantageous area from which to pounce (Tarsitano and Jackson, 1994; 1997). Tarsitano and Jackson (1997) tested six detour routes with increasing complexity and found that *P. fimbriata* could distinguish between correct and incorrect routes even when the correct route began in the opposite direction of the prey. To be able to choose the correct route, the one which led to a lure (a dead spider in a life-like posture), requires the knowledge of how all the components of the route connect to the prey, showing hunting strategies similar to those of vertebrates (Tarsitano and Jackson, 1997). This work also raises questions of mental representations in salticids, as these detours require salticids to lose sight of the prey to get to it. Despite their small nervous system, salticids are capable of surprisingly complex behaviour and impressive visual capabilities. Understanding more basic forms of learning provides an informed context to understand more complex behaviour. This is an area that has been overlooked in salticid research.

Salticids, like all arthropods, are limited in size by their exoskeleton, which also means their eye size is limited. Salticids use four separate pairs of eyes (Fig. 1.1) which, in combination, perform the tasks of high-resolution vision (high spatial acuity), motion vision, and colour vision (Land, 1971; Zurek et al., 2010; Zurek and Nelson., 2012a). These four eye pairs consist of a large pair of forward facing anterior median (AM) or 'primary' eyes and three pairs of 'secondary' eyes. Of the secondary eyes, the anterior lateral (AL) eyes are also forward facing, but smaller than the AM eyes, while the posterior median (PM) and the posterior lateral (PL) eyes, are sideways facing and smaller than both the AM and AL eyes. The main role of the secondary eyes is generally considered to be motion detection. Stimuli appearing only in the field of view of the AL eyes will mediate an orientation or optomotor response (Duelli, 1978; Zurek, 2012). This orientation response has the goal of bringing an object identified by the secondary eyes into the field of view of the high spatial acuity AM eyes for further inspection in greater detail (Zurek and Nelson., 2012b).

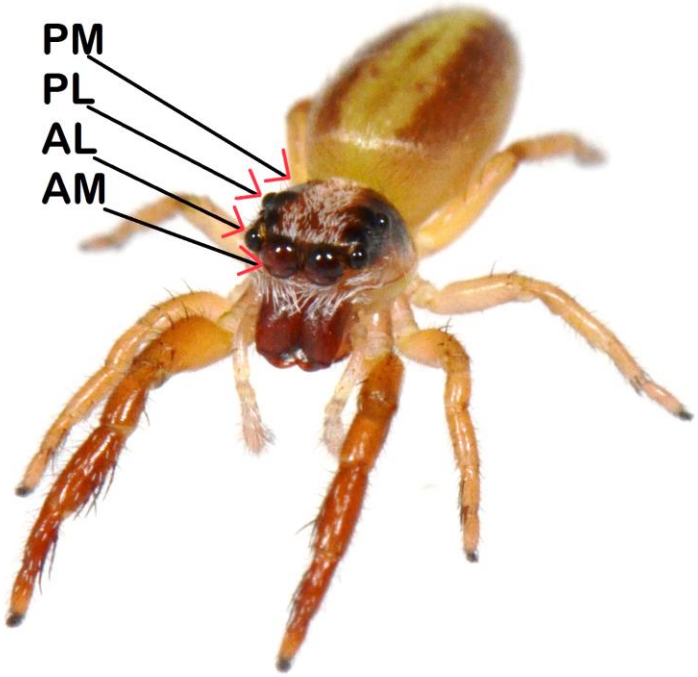

Figure 1.1. Juvenile *Trite planiceps* illustrating the posterior lateral (PL), posterior median (PM), anterior lateral (AL) and anterior median (AM) eyes.

The salticid genus *Trite* is composed of 18 species which inhabit New Zealand, Australia and the south-west Pacific (Zabka, 1988; Berry et al., 1997; Vink et al., 2011). Commonly known as the black-headed jumping spider, *Trite planiceps*, the species used for my research, is a salticid endemic to New Zealand (Forster and Forster, 1973). Apart from its mating behaviour (Jackson, 1986; Taylor and Jackson, 1999), ability to perform detours (Tarsitano and Jackson, 1994), and ability to habituate (Melrose, 2015), little is known about its behaviour.

Often found in rolled up New Zealand flax (*Phormium tenax*) and cabbage tree (*Cordyline* spp.) leaves (Forster and Forster, 1973), *T. planiceps* has a longer body (10-13 mm) than other New Zealand species of *Trite* (Vink et al., 2011). *T. planiceps*, a generalist insectivore, has a

distinguishable black head and yellow to dark green and black abdomen (Fig. 1.2). Grass moths (*Orocrambus flexuosellus*) are readily eaten by *T. planiceps* (Moss et al., 2006a), which, like other salticids, primarily use vision for capturing prey. Unlike most salticids, this species is also skilled at hunting in complete darkness in the absence of visual cues (Forster, 1982; Taylor, 1995). *T. planiceps* is parasitized by the pomplilid wasp *Epipompilus insularis*, and its known predators include conspecifics, the earwig *Forficula auricularia*, and spiders from other families, such as *Clubiona cambridgei, Cheiracanthium stratioticum* and *Zelanda erebus* (Vink et al., 2011).

Salticids are excellent study animals, especially in a laboratory setting. They are inexpensive to maintain in large numbers, and their inquisitive nature makes testing efficient and effortless. Recently, Melrose (2015) measured the orientation response of *T. planiceps* to see whether it demonstrates a response decrement to a repeated visual stimulus. Melrose (2015) determined the rate of habituation to a stimulus presented to a single AL eye compared to randomised stimuli presented to two AL eyes. The aim of my thesis is to follow-up on this work to better understand the underlying mechanisms of vigilance decrement in salticids.

Figure 1.2. Side (left) and frontal (right) view of a female adult *Trite planiceps.*

Overview of Chapters

This thesis consists of five chapters, with the introduction as Chapter 1. In Chapter 2, I introduce vigilance as background for my overall research question which was “Does the level of difficulty affect the response decrement?” I used repetitive visual stimuli of varying difficulties to test this, taking into account how motivated the spiders were. Task difficulty was manipulated by varying the contrast of the stimulus (a circle), and by varying the degree of ‘noise’ (clutter) displayed on the screen over which the stimulus moved, thus affecting the signal:noise ratio. Motivation was

manipulated by testing hungry (motivated) spiders, and sated (unmotivated) spiders. I compare the 'easy' (e.g., high contrast circle amongst low level of clutter) and 'difficult' tasks (e.g., low contrast circle amongst high level of clutter) to test whether the decrement is due to under stimulation or overstimulation. The prediction was that *T. planiceps* would respond for longer in the more 'difficult' or 'engaging' tasks if the response decrement is due to under stimulation. I predicted that hungry spiders would have a higher response rate and lower decrement than sated spiders.

In Chapter 3 I address the effect of the biological relevance of the stimuli on response decrement. Images of a circle or a fly were shown randomly to the spiders to see if they show a faster (i.e., steeper) response decrement for non-biologically relevant signals over the biologically relevant signals. The prediction of this experiment was that *T. planiceps* would show a faster response decrement to the circle than to the biologically relevant fly stimulus.

In Chapter 4 I explore whether the response decrement behaviour is controlled by the central nervous system or the peripheral nervous system. Using a stimulus of a circle amongst clutter, I used paired tests to compare the responses of *T. planiceps* when they had received a caffeine droplet, which is a central nervous system stimulant, to when they had received a water droplet (control). The prediction in this experiment was that if response decrement was a case of peripheral nervous system control, or sensory habituation, there would be no difference in the response decrement in the two groups, while if spiders were more responsive for longer when they had been given caffeine, this would indicate that the drivers of response decrement are largely controlled in the central nervous system.

Finally, in Chapter 5, I discuss my findings within a broader framework. I relate my research back to the literature and compare the results I gained from *T. planiceps* to those found in other animals in a variety of taxa.

These chapters are written in a self-contained style for publication. This entails a certain amount of repetition, especially in the Introduction section of each chapter. Repetition will be minimised in the Methods section by referring to previous chapters that describe similar methodology.

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Chapter 2

Does task difficulty affect response decrement in jumping spiders?

Introduction

Salience is known to affect the focus of attention, especially sustained attention (Itti and Koch, 2001; Helton and Warm, 2008). Sustained attention enables the maintenance of vigilance, selective attention, and continuous effort, despite changing conditions (Cohen, 2011). If a given stimulus is not salient, an animal's attention may never be driven to it, which is how camouflaged caterpillars avoid predation from birds (Lichter-Marck et al., 2015). If a stimulus is sufficiently salient, it will 'pop-out' of a visual scene and likely attract attention (Itti and Koch, 2001). Prete (1992) presented tethered praying mantises, *Phodromantis lineola* (Burr.), with two groups of 2-D worm and 'antiworm' (rectangles in which the long side was vertical) lures of different sizes against various backgrounds. Behavioural releasers were measured and defined as appetitive approaching and striking movements. Prete (1992) found that lure-to-background contrast and configuration affected prey recognition. For example, lures with low contrast were weaker releasers than those with high contrast, and lures that were darker than the background were stronger releasers. A similar phenomenon can be observed for humans in sustained attention tasks; detection probability is poorer for low salience signals compared to high salience signals and the response decrement (drop in response over time when presented with repeated stimuli) is steeper for low salience signals (Helton and Warm, 2008).

Habituation is a type of response decrement. Here, habituation is described as a behavioural decrement, in response to visual stimuli, which is due to a decrease in response within the peripheral nervous system (PNS) (Mackworth, 1968; Rankin et al., 2009), and could be thought of as local control (Rankin et al., 2009). Another type of a behavioural decrement in response to repetitive visual stimuli could be due to vigilance, defined as the action or state of monitoring the surroundings for potential threats (and in some species, mates and prey) (Beauchamp, 2015). In this case, the mechanism underlying a decrement might be under central nervous system (CNS) control.

Vigilance is recognised as a major component of anti-predator behaviour and hunting strategies (Beauchamp and Ruxton, 2012). Vigilance plays a role in the sequence of events leading to the eventual capture, in terms of both the predator when scanning for prey and the prey when scanning for predators (Endler, 1991). Vigilance can be viewed as a behaviour or state. The state, being a predisposition of the brain, cannot easily be observed directly in field studies, but the outward behavioural signs (e.g., head turning) can be observed and measured (Beauchamp, 2015). Another definition of vigilance is the probability that an animal will detect a given stimulus at a given time (Dimond and Lazarus, 1974). Detection is potentially influenced by three different classes of factors. These are the conspicuousness of the stimulus (e.g., camouflage), and the time and energy allocated to searching (e.g., prey is more likely to be detected if monitoring is more intensive, as observed in hungrier animals). Lastly, the brain itself, which processes all signals sent by the monitoring senses, may vary in its level of responsiveness to signals (Beauchamp, 2015).

There are two competing theories of the vigilance decrement in the literature; the mindlessness theory and the resource depletion theory (Helton and Warm, 2008). Imagine the following scenario. You are sitting through a seminar you have heard several times before; the content is easy to follow and it takes so little of your focus that you become understimulated and you stop paying attention. Then imagine that speaker finishes and a new one comes on discussing a topic you know nothing about and it sounds complex. The content is so difficult to follow that it takes all your focus and more so you stop paying attention again. In this case you have become overstimulated. It's the same end result, losing attention or vigilance, but the causes of that can be the polar opposite of each other. For example, with human subjects Pattyn et al. (2008) found that a response or vigilance decrement was due to understimulation or the 'mindlessness theory', whereas Helton and Warm (2008) found support for the overstimulation or 'resource depletion theory'.

If the response decrement observed in invetebrates can coincide with the understimulation theory in humans, then there should be a faster response decrement in tasks that are easier to process. This is based on the assumption that there is too little cognitive load from an easy stimulus, so the attentional system loses interest and withdraws effort (Pattyn et al., 2008). Non-responsiveness in a simpler system could possibly be interpreted as an adverse state caused by a foraging 'patch' being unproductive (Beauchamp and Ruxton, 2012). However, if the response decrement observed coincides with the overstimulation theory in humans then there should be a faster decrement in tasks that are cognitively difficult to process (e.g., low salience, high clutter),

based on the assumption that the psychophysical contrast is so challenging that it potentially burns up the replenishable cognitive resources required for paying attention (e.g., overworked neurons Helton et al., 2002).

Jumping spiders (Salticidae) are comparatively small spiders (2-10 mm as adults), with short legs and a broad, cephalothorax holding large eyes. They typically do not use webs to catch prey, but instead use silk to build nests in which to rest and lay eggs (Foelix, 2011). Salticids are persistent hunters and are very attentive to moving stimuli; after turning to face an object, they will move closer if it is perceived to be of interest, and in the case of potential prey, slowly stalk it and ultimately pounce (Foelix, 2011). Salticids have four pairs of eyes which combined have fields of view extending almost 360 degrees (Land, 1985). The forward-facing pair of 'principal' eyes (PL) have narrow fields of view, but combine excellent spatial resolution with colour vision. In addition, three pairs of 'secondary' eyes around the cephalothorax provide wide fields of view and function primarily as motion detectors (Duelli, 1978; Zurek and Nelson., 2012a). Of these, the anterior lateral (AL) eyes, is the largest and is also forward-facing. Motion detected by the secondary eyes may elicit an orienting or optomotor response, whereby the salticid rapidly turns to face the object to examine it further using the high-acuity principal eyes.

Salticids display some of the richest behaviours among arachnids (Wilcox et al., 1996; Harland and Jackson, 2004; Jackson and Harland, 2009) despite having roughly 500,000 neurons (Land M. pers. comm. with XJN). However, it is their predatory behaviour that has received the most attention (reviewed in Jackson, 1996b; Nelson and Jackson, 2011). To be successful hunters, salticids must attend to a wide range of visual stimuli, but be selective whilst doing so (Prete, 2004). Looking for prey requires complex pattern recognition, which is a computationally demanding task (Dukas, 2002). Salticids and other animals must restrict the amount of visual information that is processed at any given time (Broadbent, 1965; Milinski, 1990; Dukas, 1998; Kastner and Ungerleider, 2000). For example, the field of view for most animals (180^{o} to 360^{o}) is much larger than the subset to which it is beneficial to focus (Dukas, 1998). Selective attention allows animals to respond selectively to competing stimuli, enabling some stimuli to evoke a behavioural response while others are ignored (Blake and Logothetis, 2002; Bichot et al., 2005; Paulk et al., 2014). Even animals with small brains are capable of selective attention (Sztarker and Tomsic, 2011; Paulk et al., 2014), such as dragonflies capturing flies in swarms comprised of both prey and conspecifics

(Corbet, 1999). Selective attention can affect diet choice and constrains the ability to feed and simultaneously attend to predators (Dukas, 2002).

Previous work has shown that *Trite planiceps*, the salticid species used in this study, stops responding to repetitive visual stimuli (Melrose, 2015). It was initially suggested that the response decrement observed in *T. planiceps* was due to habituation, but conclusions could not be made on those data alone (Melrose, 2015). Here, my aim was to determine what effect task difficulty (e.g., the salience of stimuli) has on the response decrement to repetitive visual stimuli in *T. planiceps*. Task difficulty was manipulated by varying the contrast of the stimulus (a circle), and by varying the degree of 'noise' (clutter) displayed on the screen over which the stimulus moved, thus affecting the signal:noise ratio. Motivation was manipulated by testing hungry (motivated) and sated (unmotivated) spiders in a paired fashion. I compared the 'easy' (e.g., high contrast circle amongst low level of clutter) and 'difficult' tasks (e.g., low contrast circle amongst high level of clutter) to test the driver(s) of the response decrement. I predicted that if *T. planiceps* is demonstrating a response decrement due to under stimulation, the spiders will show a faster decrement in the 'easy' tasks and a slower decrement in the 'hard' tasks.

Materials and Methods

Housing

Lighting in the lab was on a 12:12 L:D cycle commencing at 0700 h. Temperature was kept constant at 25^{o} C. Adult *Trite planiceps* were housed in upside down transparent plastic jars (17 cm tall x 8 cm wide) with a cotton roll inserted in a hole in the bottom of the lid. The cotton roll hung into a cup of water to ensure constant humidity. Two additional holes were drilled into the opposite end of the jar; one covered with mesh for ventilation and the other plugged with a cork that could be removed for inserting houseflies (*Musca domestica*) or *Drosophila* as food, which were given to each spider once per week. Housing jars were cleaned weekly. Inside the jar was a folded piece of card providing environmental enrichment in which *T. planiceps* could hide and build nests.

Apparatus

The apparatus, or viewing rig, consisted of a holding stand, a Logitech® webcam, and two identical monitors placed at a 120^{o} angle from each other (Fig. 2.1). The monitors were 17'' Phillips Brilliance 170P 1280 x 1042 with a refresh rate of 75 Hz. The monitors were standardised for brightness and colour using Spyder 4 Pro® colorimeter and accompanying software. Stimuli crossed on the horizon of the spider's field of view (i.e., 0^{o} vertically) and moved from posterior to anterior. Stimulus

presentation was separated by a 10 s inter-stimulus interval (ISI) and the stimulus was presented 80 times for each salticid. For this experiment, the stimuli were grey circles subtending 1.3° from the point of view of the test subject (40-pixel diameter) and which were presented amongst a cluttered background. The group 1 stimulus was set at 10% opacity (RGB 239, 239, 239), and opacity for group 2 was 30% (RGB 213, 213, 213). Stimuli were created in Adobe Premiere CC® 2015, as described below.

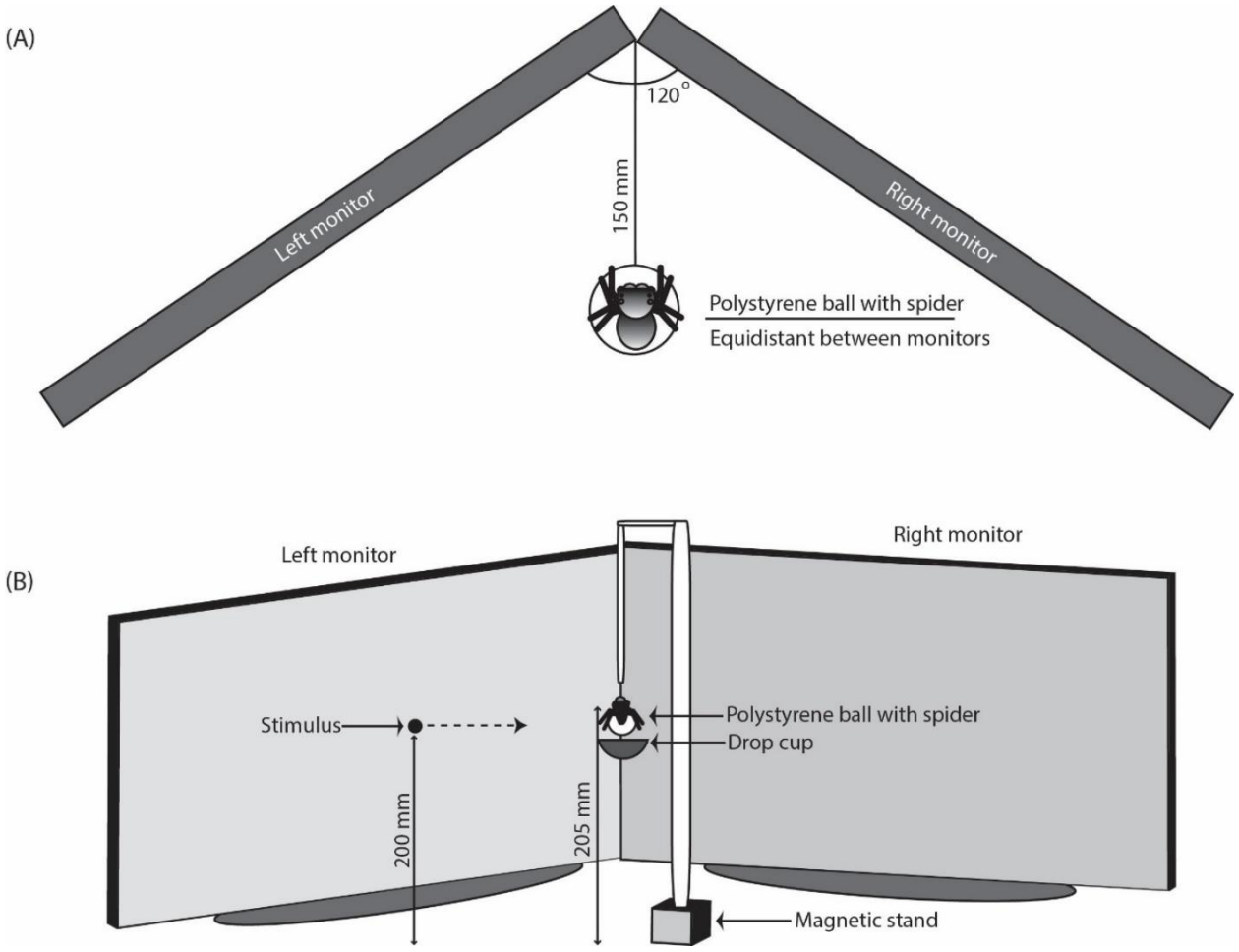

Figure 2.1. Apparatus used for *Trite planiceps* tests. (A) bird's-eye view of the position of the salticid on the polystyrene ball. (B) Frontal view demonstrating the position of the rig holding the salticid. The drop cup catches any dropped balls which are close enough for the salticid to retrieve. A small platform supports the drop cup (not shown).

To generate a stimulus within Adobe Premiere®, a full stop was enlarged to 40 pixels and opacity was changed to 10% for the low contrast stimulus circle and to 30% for the high contrast stimulus. Each video was made as follows: Premiere® was configured to display media at the screen size of the apparatus (i.e., 2560 x 1042 pixels, which was the combined size of the two monitors).

Two motion files were generated, one for each screen. The stimulus moved on screen from 13 cm to 8 cm, from the centre point of the screen toward the intersection of the two screens (i.e., posterior to anterior). For this species, Melrose (2015) demonstrated that, to coincide with the field of view of the AL eyes, stimuli needed to appear within 8 - 16 cm (321 - 640 pixels) from the centre (midpoint) of the monitor (Fig. 2.2). The stimulus was then set to change to 0% opacity (effectively disappearing) once it reached the 8 cm mark, and continued moving at 0% opacity for 10 s to create the ISI. This generated a 15 s video with the circle moving for 5 s with a 10 s ISI.

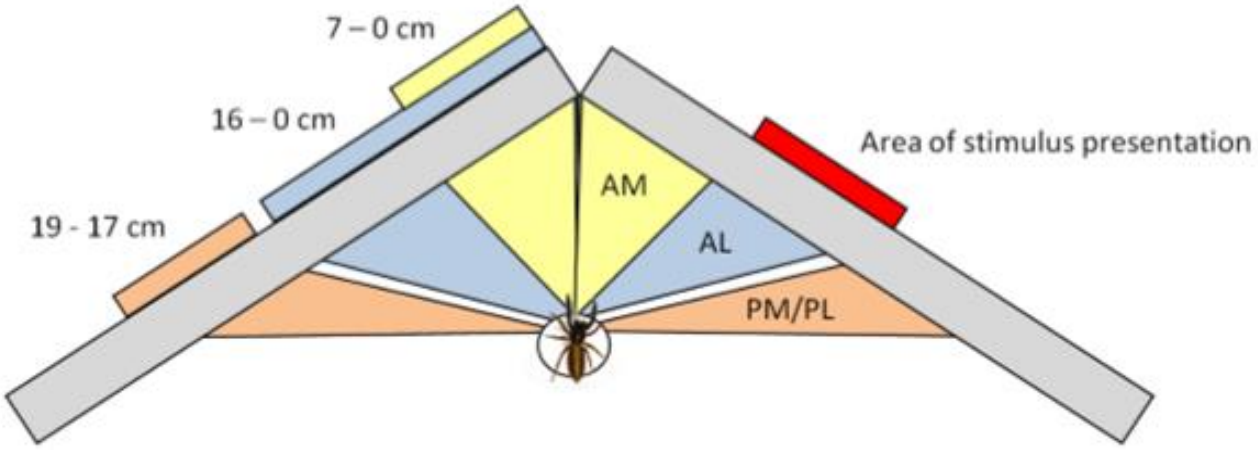

Figure 2.2. Aerial view of the field of view of the salticid that elicited an orienting response for each eye pair, as determined by a stimulus displayed against a grid display on each computer monitor (shaded grey). Eyes are coded as: AM (anterior median), AL (anterior lateral), PL (posterior lateral) and PM (posterior median). Red area indicates where the stimulus was presented during testing based on Melrose (2015) preliminary grid tests.

Task difficulty was manipulated by presenting each stimulus amongst backgrounds of different levels of clutter, which were created separately. Clutter was generated using Adobe Photoshop® CS6 and later exported in to Adobe Premiere® CS6 Motion Effects to generate unique movie files combining each level of clutter and either high contrast (30%) or low contrast (10%) stimuli. Clutter was added as a foreground image with the moving stimulus set as the background layer. The output was set to 2560 x 1042 pixels (the combined size of both monitors) and had a grey background (RBG 250, 250, 250), as used in previous experiments (Melrose, 2015). To make clutter, the noise filter in Photoshop® was used to assign random pixels to the image, which allowed for manipulation of the amount of noise, type of distribution, and colour mode. I used a Gaussian distribution and monochromatic colour mode for all clutter levels. I used four levels of clutter on a doubling scale, starting at 20%, followed by 40%, 80% and 160% (the scale in Photoshop® goes up

to 400%, so 160% does not create a solid background). Preliminary tests were conducted to determine both the lowest opacity for the circle and the maximum amount of clutter needed to produce a ceiling effect, which was defined as the point at which half of the test spiders responded half of the time to the 10% circle against the 160% level clutter.

Luminance, brightness and RGB values for each clutter level were measured within Photoshop® by averaging the whole image (Table 2.1). I standardised the monitors and therefore the appearance of the stimuli (circles amongst clutter) by setting the viewing characteristics of the computer screen used for presentation to be the same as the screen used to create stimuli (100% brightness, 50% contrast and 6500K colour).

Table 2.1. Characteristics of clutter levels (top) and stimuli (bottom) used in experiments on *Trite planiceps*. For clutter, the level percentage and for stimuli, the contrast, luminance, brightness and RGB values were measured within Photoshop®. Weber contrast measured as $I_{stimulus} - I_{background}/I_{background}$.

Clutter level (%)	**Luminance (cd/m2)**	**Brightness (%)**	**RGB**	**Weber contrast**
20	92	91	232, 232, 232	-0.061
40	85	83	211, 211, 211	-0.133
80	74	71	182, 182, 182	-0.245
160	66	63	160, 160, 160	-0.327
Stimulus contrast	**Luminance (cd/m2)**	**Brightness (%)**	**RGB**	**Weber contrast**
10	94	94	239, 239, 239	-0.041
30	85	84	213, 213, 213	-0.133

Testing protocol

Each stimulus was presented on either the left or right screen as determined by a random number generator (https://www.random.org/). Videos were exported as 21 min clips (80 trials, with 1 min leeway) using an HEVC (H.265) codec and were presented to the spiders using VLC media player 2.2.2. The stimuli were presented so that they were only visible to the AL eyes.

Female *T. planiceps* adults and sub adults (N=19) were used (male salticids are considerably less responsive toward stimuli than females; (Zurek and Nelson., 2012a). Spiders were randomly split into two groups, of ten and nine, for testing. One group was shown the high contrast stimulus,

and the other was shown the low contrast stimulus amongst different levels of clutter. Testing was carried out between 0700 to 1300 hours.

Before testing, salticids were removed from their jars and were placed inside the 'gunkatron'. This was composed of a cylinder that was open at one end, with holes drilled in the opposite, closed, end. Another cylinder, capped with foam onto which a spider was placed, was sized such that when it was plunged into the first cylinder, the spider's cephalothorax could be accessed through the holes. This permitted me to harmlessly restrain each spider. Once in position, the spider was fixed to a TPC® disposable micro applicator (termed the 'dental stick') by applying beeswax to the top of the head (Fig. 2.3). The dental stick with the spider attached was then suspended within the testing apparatus by a crocodile clip (Fig. 2.1). The spider was given a 17 mm crosshair marked polystyrene ball (weight 150 mg) to hold on to. This weight is easily held by sub adult and adult female *T. planiceps* (mean spider weight 53.26 (± 3.39 SEM) mg, n = 23) for several hours without evidence of fatigue (see Zurek et al., 2010). Within the rig, 10 mm underneath the polystyrene ball was a small concave 'cup' which served to catch the ball in case the spider attempted to jump and dropped the ball (Fig. 2.1). The ball was cleaned with 70% ethanol after each spider was tested to remove any chemicals deposited by previous individuals.

Figure 2.3. A *Trite planiceps* female holds on to a marked polystyrene ball whilst being suspended from a dental stick attached with beeswax.

Once attached to the apparatus, the spider was equidistant (at 150 mm) from both left and right stimulus presentation monitors (Fig. 2.1). Preliminary data had already established that there is no bias for left or right screens (see Chapter 3; Melrose, 2015). After being suspended in front of the apparatus, the salticid was given 20 min to settle from handling stress before being presented with a stimulus movie.

When presented with a visual stimulus, the orienting response of the salticid causes the polystyrene ball that it is holding to rapidly swivel in the opposite direction to the stimulus (Zurek and Nelson., 2012a). I categorised and recorded several responses. These were: 0 for 'no movement'; 1 for 'general activity', such as grooming, which was recorded to control for the possibility that any response decrement observed was not simply a freezing response or fatigue; 2

were ‘fast responses’ towards the stimulus, but not at optomotor speed (see 3); 3 were ‘optomotor responses’, which are exceedingly fast whole body swivels (c. 700°/s) whereby the spiders (attempt to) turn towards the stimulus. These are highly characteristic of salticids and are unmistakeable.

Each salticid was randomly placed into group 1 (30% circle) or group 2 (10% circle). Each salticid in groups 1 and 2 was exposed to every level of clutter for both high (30%) and low (10%) stimulus contrast. Presentation order was randomised using a random number generator (https://www.random.org/) to prevent learning effects. Tests were conducted when salticids were hungry, having been without food for 6-8 days, and when they were sated, having eaten the previous day. Overall, each spider was tested eight times (4 levels of clutter X 2 levels of hunger).

Experimental design and statistical analyses

The experiment consisted of a full-factorial design comprising stimulus type (light or dark circle), background clutter level (1, 2, 3, 4) and hunger (hungry or sated) for each experimental unit (spider). Each spider was subject to one stimulus type, stimuli consisted of circles that were either low or high contrast, across all four levels of clutter, when sated and when hungry, resulting in a total of 8 tests per individual. Treatment combinations were assigned at random. All analyses were conducted using the program R 3.3.1 (R Core Team, 2016). The proportional odds model predicted the probability for a spider to fall into a specific response category across 80 trials. Data were analysed with a cumulative link mixed-effect model, assuming a logit link function, since the data were categorical (Crawley, 2007). Individual differences between spiders were taken into account through random effects included in the model; assuming different intercepts (threshold parameters) and trial slopes for each spider. Model selection was used to determine an appropriate fixed effects structure (containing the first term and second order interactions). Potential models were compared and ranked through the ‘AICcmodavg’ package within R© that selects the ‘best’ model based on Akaike's Information Citerion (AIC) (Akaike, 1973; R Core Team, 2016).

For visualisation, data were fitted with an exponential function $f(t) = \alpha e^{\lambda t}$ over 80 trials in MatLab© version 2015a. Here, λ is the decay rate, α is the initial quantity of the response, and t is the current trial. For this, I omitted the ‘no movement’ category (category 0) and merged the ‘fast response’ and the ‘very fast response’ (categories 2 and 3). Consquently, I plotted the exponential function of ‘activity’ (response category 1) and of ‘interest’ (response) toward the stimulus in order to disambiguate any decrement in response toward the stimulus from general fatigue (activity).

Results

My analysis showed the probability of a spider movement to fall into a certain response category over 80 trials (i.e., determining a slope for the response decrement). Full model selection and a summary of all coefficient outputs can be found in Appendix 1. Once ranked, the set of models were restricted to pairwise interactions only, based on prior knowledge of the nature of the data. Thus, Table 2.2 only includes models with 2-way interactions or less.

Table 2.2. Model-selection results for evaluating clutter levels and stimulus salience on responses to repetitive visual stimuli in *T. planiceps* (N=19). Effects included are the fixed effects of interest and their pairwise interactions. K is the number of estimated parameters for each model, AICc is Akaikes Information Criterion, Δ AICc is the appropriate delta AIC component. AICcWt is the Akaike weights.

Effects included	**Model**	**K**	**AICc**	**Δ AICc**	**AICcW**
All terms	(T+D+H+C)^2	24	24623.66	24.48	0
All terms excluding stimulus effects	(T+H+C)^2	18	24666.01	66.83	0
All terms excluding hunger effects	(T+D+C)^2	18	24764.16	164.98	0
Trial, clutter	(T+C)^2	13	24806.39	207.21	0
Trial, hunger	(T+H)^2	9	24837.13	237.95	0
All terms excluding clutter effects	(T+D+H)^2	12	24839.63	240.45	0
Trial	T	7	24903.77	304.59	0
Trial, stimulus	(T+D)^2	9	24905.15	305.97	0

There were four main effects of interest: T = trial, D = stimulus type, H = hunger level, C = clutter.
^2 indicates a pairwise interaction between effects

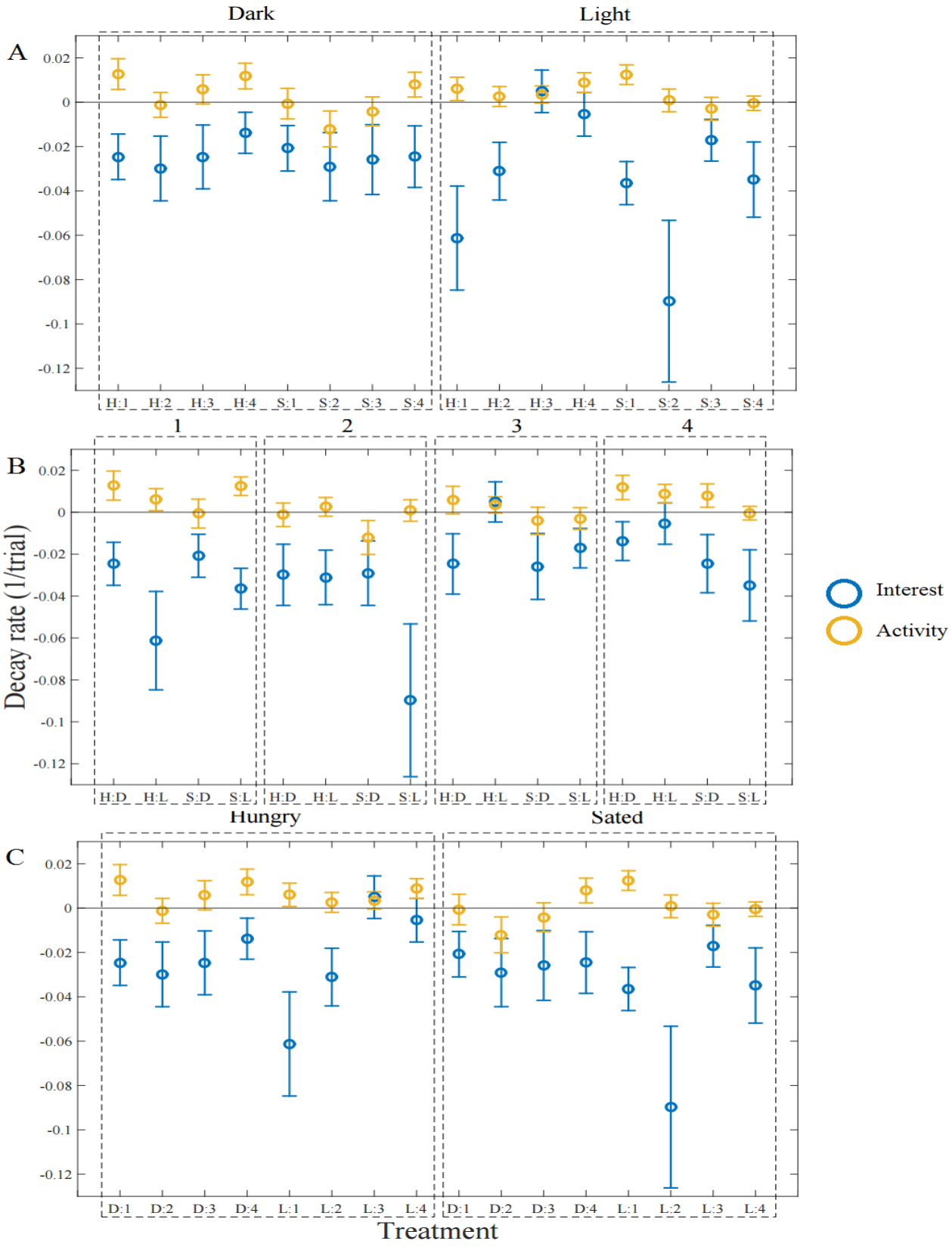

Figure 2.4. Response decay rate in *Trite planiceps* fitted as an exponential function over 80 trials. Spiders, when 7 days hungry ('H' on X-axis in panels A and B, left on panel C) and sated ('S' on X-axis in panels A and B, right on panel C), were shown repetitive visual stimuli of a 40-pixel dark (left on panel A, 'D' on X-axis in panels B and C) or light (right on panel A, 'L' on X-axis in panels B and C) circle against four levels of clutter (1 low – 4 high on panel B, depicted as 1-4 X-axis in panels A and C). Yellow depicts general movement, while the blue represents distinct responses (combining optomotor and fast responses) toward the stimulus. Note: data across panels A, B and C are the same, but presented differently to facilitate interpretation.

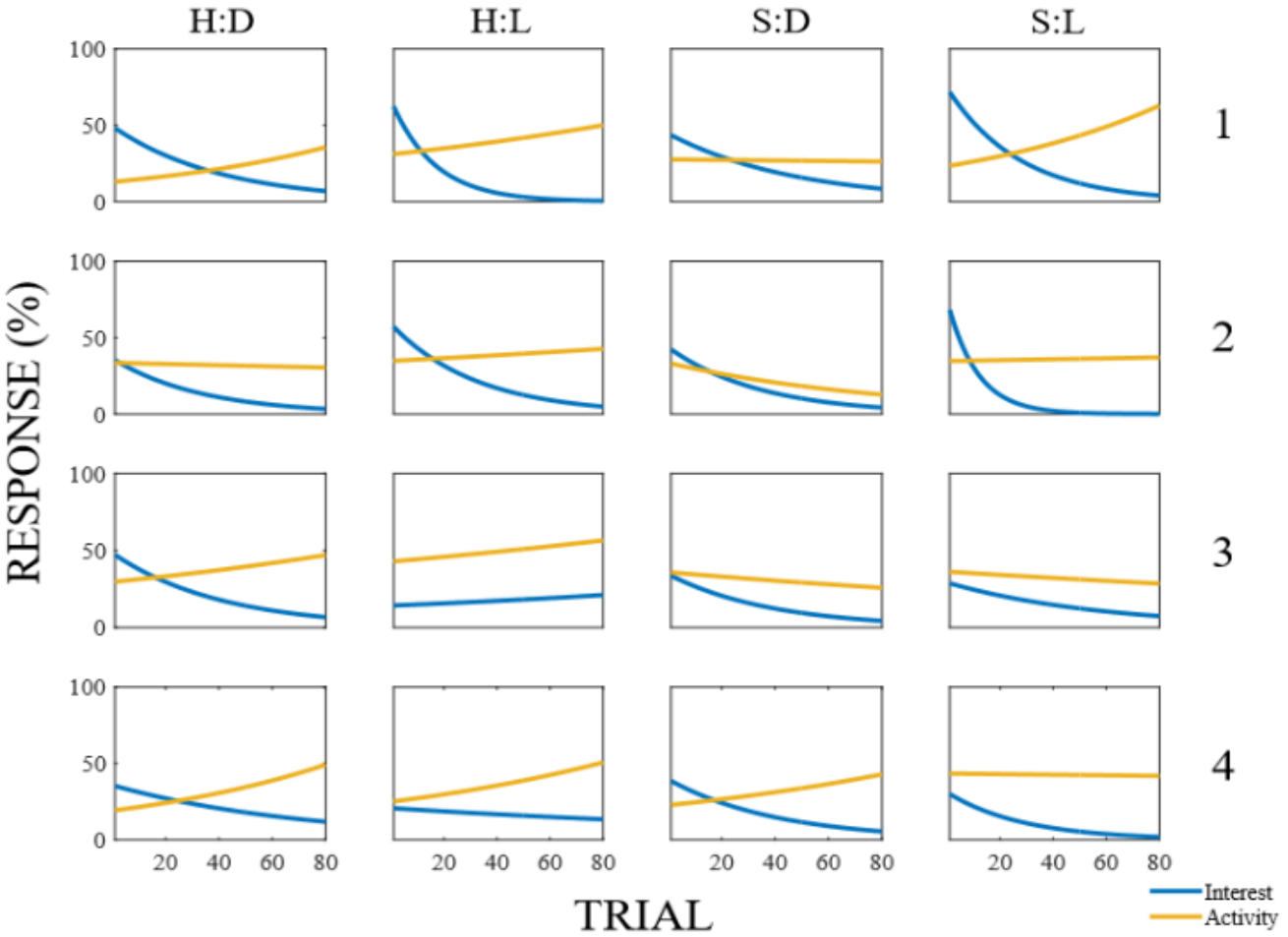

Figure 2.5. Exponential regression of the responses in *Trite planiceps*. Spiders, when 7 days hungry (depicted as 'H') and sated ('S'), were shown repetitive visual stimuli of a 40-pixel dark (depicted as 'D') or light (L) circle against four levels of clutter (1 low – 4 high, on right). The yellow lines depict general movement, while the blue lines represent distinct responses (combining optomotor and fast responses) toward the stimulus. Note: data for this figure is based on that used for Fig. 2.4.

Generally, the best model showed significant effects of trial, hunger, clutter level and stimulus contrast (Table 2.2). However, these data were difficult to interpret due to the high number of variables. In the dark circle condition, there was less variability in the rates of decay (between c. 0.03 and -0.01 per trial) irrespective of hunger or clutter level, compared to the light circle condition (between -0.09 and 0.01 per trial; Fig. 2.4A). All conditions showed a response decrement except in clutter level 3 with the light circle when hungry, which showed a slightly positive slope (0.005; Figs. 2.4 and 2.5). Across both stimulus types, interest decreased steeply in the low (levels 1 and 2) clutter conditions (mean decay rate =-0.040) and the baseline levels were higher (c. 56%) compared with higher clutter (levels 3 and 4; Fig. 2.5). In the high clutter condition, baseline responses to both stimuli were low (c. 32%), and consequently the slopes were more gradual (mean decay rate = -0.018).

In the low clutter conditions (levels 1 and 2) the dark circle stimulus showed lower baseline responses (c. 43%) than the light circle (c. 69%; Fig. 2.5), but the dark circle elicited a more gradual response decrement (mean decay rate = -0.026) compared with the light circle (mean decay rate = -0.055).

Hunger effects appeared to be minimal in the dark circle task (Fig. 2.4). The trends for the light circle in the lowest level of clutter when hungry (-0.061) and when sated (-0.036) were slightly opposing, whereas the decay rates for the dark circle in the same conditions were very similar (-0.025 when hungry and -0.021 when sated).

Discussion

This study tested the effect of difficulty on response decrement in salticids, taking into account the motivation (hunger) level of the test subject. In the light circle (low salience or 'difficult') condition, there was more variation than in the dark circle (high salience or 'easy') condition, probably because the task was more challenging. All conditions but one revealed a response decrement in *T. planiceps*; level 3 clutter with the light circle showed a positive decay rate which is difficult to explain. In both low and high salience tasks, interest decreased steeply in the low clutter levels. This is unsurprising given that the baseline of responses was higher at the beginning of trials; in the high clutter level tests, baseline responses to both the low and high salience stimuli were low, where it seems likely that the task was simply too difficult. The initial low responses could make it appear that the response decrement was more gradual (i.e., spiders were 'interested' for longer) but the slope observed could just reflect individual differences. From personal observation during testing, it was noted that a few spiders responded consistently throughout the high clutter levels, while others did not respond at all. Because of this observation and the preliminary tests which found a ceiling effect in the high clutter levels, for the remainder of this discussion I will interpret results from the low clutter levels (the light circle in low clutter, levels 1 and 2, is still a difficult task).

In the high salience condition, baseline responses were lower than in the low salience condition but revealed a shallower decrement. In the low salience condition the spiders were more responsive at the start but then responses decayed faster. The high baseline responses to the low salience stimulus are likely due to the spiders taking more attempts to identify the stimulus because when the circle was harder to see (low salience), the clutter obscuring the stimulus would have made it more difficult to disambiguate than when the circle was easier to see (high salience).

Despite the high spatial acuity of the AL eyes (Zurek et al., 2010), it seems very likely that it would take more effort to disambiguate the stimulus that was not as salient. However, in the high salience condition, it may be that *T. planiceps* could determine quite quickly that the stimulus was not biologically relevant. This would explain the low baseline responsiveness. However, the responses progressed differently across time, with the response decrement to the low salience stimulus being steeper than the high salience stimulus in the latter trials.

While statistically significant, hunger effects appeared to be relatively minimal, especially in the high salience task. In the low salience task, hunger (motivation) had more of an influence, but did not appear to factor into the differences in response decrement. Overall, there were a lot of interactions in this experiment. There were two salience stimulus levels, four clutter levels and two motivation levels, which made interpreting these data challenging. There was no evidence of fatigue in any condition, so we can conclude that any response decrement was not due to spiders becoming tired. Additionally, through both personal observation and statistical analysis of this experiment, it was apparent that *T. planiceps* demonstrated fewer optomotor (exceptionally fast or 'urgent') responses than fast responses (see Appendix 2). This could be due to the clutter occluding the circles or to the relatively low contrast (maximum 30%) of the circles. Previous work (Zurek and Nelson., 2012a) has shown that there is an ideal level of contrast (about 40%) to elicit high rates of orientation responses in salticids.

Reducing the number of clutter conditions would allow spiders to be tested 8 times overall (2 dot levels X 2 clutter levels X 2 levels of hunger) by either eliminating the medium clutter conditions (i.e., levels 2 and 3) with just the extremely hard and extremely easy levels remaining, or by eliminating the two high clutter conditions (i.e., levels 3 and 4). It would then be easier to test all spiders in all conditions (i.e., paired testing for both circle contrasts). Spiders in this experiment showed high mortality rates towards the end of being tested eight times, indicating a limit for enduring this amount of testing in a relatively short time frame (c. 40 days). The high mortality rate could have been due to handling stress, or something test-related, such as cognitive demand over extended periods.

Behavioural tests in humans suggest that there is a higher probability of detecting a single stimulus (selective attention) than detecting multiple stimuli (divided attention) (Moran and Desimone, 1985; Heinze et al., 1994; Spitzer et al., 1998; Kastner and Ungerleider, 2000). For example, Corbetta et al. (1990) presented participants with two successive images that differed

slightly in shape, colour or speed and found that subjects performed better when informed which attribute would be different than when they were told the images could differ in any of the three attributes. Dukas (2004) reviewed evidence for differences in selective versus divided attention in other species. Clear evidence to support this comes from laboratory studies of blue jays (*Cyanocitta cristata*) (Dukas and Kamil, 2001). Blue jays were trained to search for and peck at two types of targets presented at random locations on computer monitors that recorded peck locations. Targets were presented amongst a background of randomly placed items in a way that made two target types cryptic. The authors compared the overall target detection rate when the birds either focussed attention on searching for one prey type or divided attention between two prey types, finding that target detection rates were much lower when the birds had to divide their attention than when they could focus on one prey type (Dukas and Kamil, 2001).

Limited attention lowers the probability, for both foragers and hunters, of detecting cryptic food items. Animals may be less likely to notice an approaching predator while engaged in an attentionally demanding task (Milinski, 1990; Krause and Godin, 1996; Dukas, 2002). A larger attentional capacity (i.e., a larger volume of neural machinery devoted to processing information) should enable better detection and more refined perception. However, this would require larger neuronal tissues dedicated to visual detection and consequently would entail larger metabolic expenditures (Dukas, 2002; Dukas, 2004). The results I found in this chapter may suggest that *T. planiceps* was initially selectively attentive to the low salient stimulus but as it became more difficult, and thus more cognitively demanding, their responses tapered off faster.

Insects such as *Drosophila* (Poggio and Reichardt, 1976; Heisenberg and Wolf, 1984; van Swinderen and Greenspan, 2003; Sareen et al., 2011), dragonflies (Olberg et al., 2007), and praying mantises (Rossel, 1980) respond selectively to salient visual stimuli, but it has not often been clear where the attentional selectivity occurs in the insect brain (Paulk et al., 2014). Paulk et al. (2014) investigated the neural correlates of visual attention in honeybees (*Apis mellifera*) using a closed-loop paradigm. The paradigm allowed tethered bees to select and fixate on visual cues by rotating an air-supported ball in a virtual reality arena. The authors found that the honeybee fixation behaviour increased stimulus-specific brain activity when the bees were presented with competing objects, such as two green bars instead of one. When the bars were closer together (30^{o} or 60^{o} apart) bees fixated between the competing bars, whereas when the bars were further apart (90^{o} – 180^{o}) the bees alternated fixation on either bar (Paulk et al., 2014). Paulk and colleagues (2014) also found that the attention-like processes in honeybees are pushed far out into the sensory

periphery, as demonstrated by selective attention in the optic lobes preceding the behavioural choices in honeybees.

The response decrement observed in *T. planiceps* was not likely due to habituation. Full cessation of responses was not observed except in one condition, and the slope of responses changed with different difficulty, indicating some level of processing was occurring. Additionally, an important pre-requsite for sensory habituation is that, when given a dishabituating stimulus, the animal regains normal response levels (Thompson and Spencer, 1966), yet this does not happen in *T. planiceps* (Melrose, 2015). Instead, the observed response decrement is most likely a form of vigilance decrement. There are two theories prevalent in the literature, both of which were developed regarding human vigilance: the understimulation or mindlessness theory (Pattyn et al., 2008) and the overstimulation or resource depletion theory (Helton and Warm, 2008). The understimulation theory proposes that easy tasks take little focus and are not cognitively demanding, so the attentional system loses interest and withdraws effort. For example, Pattyn et al., (2008) investigated the time on task during tedious monitoring tasks and found that both physiological and subjective experiences of the human subjects revealed the vigilance decrement was due to understimulation. If the response decrement observed in invertebrates resembles the understimulation theory, there should be a faster response decrement in tasks that are easier to process (Stuss et al., 1995; Robertson et al., 1997; Manly et al., 1999), as I predicted in this study (i.e., a faster response decrement to the easiest - high salience, low clutter - task). However, responses in *T. planiceps* did not coincide with the understimulation theory. Instead, I found a greater decrement in the harder task (low salience, amongst clutter). Psychological studies, in both humans (Grier et al., 2003; Helton et al., 2005; Helton and Warm, 2008) and animals (Dukas and Clark, 1995; Beauchamp and Ruxton, 2012) have established that the central nervous system cannot sustain vigilance for an extended period of time (Nuechterlein et al., 1983; Warm, 1984; Parasuraman and Mouloua, 1987). The overstimulation vigilance decrement studies in humans have described similar results to those described here. For example, Helton and Warm (2008) found that detection probability was poorer for low salience signals compared to high salience signals and the vigilance decrement was steeper for low salience signals. This is what was observed in *T. planiceps*; they responded more at the beginning of trials to the low salience stimulus (likely because it was harder to initially disambiguate) and responses then tapered off faster than they did in the high salience condition. Based on modelling, Dukas and Clark (1995) found that the rate of vigilance decrement was faster for more difficult tasks. The model incorporated the parameters of

vigilance decrement during foraging and vigilance recovery during rest and results showed that both the total time spent foraging and the duration of each foraging episode was small for more demanding foraging activities. The consequent vigilance decrement implied a gradual reduction in the ability to process information effectively (Dukas and Clark, 1995). It is possible that sustaining attention to the stimulus that was harder to see (the low salience task described here) over about ten trials used up the replenishable cognitive resources required for such attention, as suggested for humans (Helton and Warm, 2008). Overall, the overstimulation theory seems a more reasonable hypothesis to explain response decrement in salticids.

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Chapter 3

Does biological relevance affect the behaviour of jumping spiders?

Introduction

Survival and reproduction requires that animals find food and mates, and avoid predators. It makes sense, therefore, that natural selection would favour animals who are attentive to items that could be prey, mates, or predators (Prete, 2004) and it is found that these items tend to be perceived more readily than those that are not (Bond and Kamil, 2002; Schülert and Dicke, 2002; Dolev and Nelson, 2014). For example, humans and other primates are extremely visually sensitive to snakes (Kawai and He, 2016). Many snakes use camouflage to conceal themselves from both prey and their own predators, making it very challenging to detect them. However, in a series of rapidly presented images, Kawai and He (2016) showed that humans can detect pictures of snakes more quickly than those of other animals under difficult visual conditions, probably because snakes have acted as a selective pressure on primate visual systems. A similar phenomenon can be observed in jumping spiders (Salticidae). Menda and colleagues (2014) presented salticids with different prey or non-prey images and recorded extracellular responses from neurons in the central nervous system. The images used were dorsal and lateral views of a fly (potential prey), a conspecific salticid (potential mate or rival) and a known control (Tanaka et al., 1993; Tsao et al., 2003; Sheehan and Tibbetts, 2011) which was a scrambled fly image, retaining the size and contrast features of the fly while destroying figural integrity. They found that the response rate to the intact dorsal fly image was significantly different and greater than the response to the scrambled fly image (Menda et al., 2014). These studies show that biological relevance can alter how animals respond to visual stimuli, from salticids through to humans.

Salticids are small spiders (2-10 mm as adults), with short legs and a square prosoma that holds large eyes. Salticids do not use webs to catch prey, but instead, use silk to build nests in which to lay eggs, rest and protect themselves against weather (Foelix, 2011). Salticids are cursorial hunters and are very attentive to moving stimuli; after a quick turn to face an object, and in the case of potential prey, slowly stalk it and ultimately pounce (Foelix, 2011). Salticids have a

division of labour-type visual system comprising four pairs of eyes that surround the head giving a view of almost 360 degrees (Land, 1985). The forward-facing pair of 'principal' eyes have narrow fields of view, but combine excellent spatial resolution with colour vision, while the three pairs of 'secondary' eyes have wide fields of view and function primarily as motion detectors (Duelli, 1978; Zurek and Nelson., 2012a). Of these secondary eyes, the anterior lateral (AL) eyes are also forward-facing, while the two remaining pairs are found along the side of the head. Motion detected by the secondary eyes may elicit an orienting response, at which point the object of interest is inspected further using the high-acuity principal eyes. Salticids have a small number of neurons (roughly 500,000; Land M. pers. comm. with XJN) yet display some of the most complex behaviour among arachnids (Wilcox et al., 1996; Harland and Jackson, 2004; Jackson and Harland, 2009).

To be successful hunters salticids must pay attention to a wide range of visual stimuli, but be selective whilst doing so. Looking for prey requires computationally demanding complex pattern recognition (Dukas, 2002). Salticids and other animals must restrict the amount of visual information that is processed at any given time (Broadbent, 1965; Milinski, 1990; Dukas, 1998; Kastner and Ungerleider, 2000). For example, the field of view is generally 180^{o} to 360^{o} for most animals, but prey may only cross 30^{o} of that space (Dukas, 1998). Selective attention describes an animal's ability to respond selectively to competing stimuli, enabling some stimuli to evoke a behavioural response, while others are ignored (Blake and Logothetis, 2002; Bichot et al., 2005; Paulk et al., 2014). Selective attention can affect diet choice and can constrain an animals' ability to simultaneously feed and attend to predators (e.g., searching for cryptic prey in an open-to-predators area; Dukas, 2002). Even animals with the smallest brains are capable of selective attention (Sztarker and Tomsic, 2011; Paulk et al., 2014). For example, dragonflies capture flies in swarms comprising both prey and conspecifics (Corbet, 1999).

The famous phrase 'jack of all trades' is typically followed by 'master of none'; in the case of salticids in the genus *Portia,* however, it seems these may be the 'jack of all trades, and master of all' (Jackson and Hallas, 1986; Jackson, 1996a; Harland and Jackson, 2004). As araneophagic (spider-eating) hunters, *Portia* employs many techniques to catch prey (jack of all trades) and all of them are relatively complex (master of all). For example, when hunting, *Portia* uses smokescreen behaviour to mask stalking movements (Wilcox et al., 1996), aggressive mimicry (Jackson and Wilcox, 1993) and deceit (Jackson, 1992), as well as performing detours away or out of sight from a food source (Tarsitano and Jackson, 1994; 1997). *Portia* is 'clever' but slow at what it does (Jackson,

1992; Harland and Jackson, 2000), perhaps because of some neuronal processing limit (i.e., has complex behaviour by compromising on speed). Nevertheless, studies on orb weavers have shown that miniaturisation does not compromise behavioural complexity (Eberhard, 2007; 2011), at least not for innate behaviours such as web building. Another possibility is that *Portia* moves slowly because this more closely resembles detritus in a web, which it is thought to mimic (Jackson and Blest, 1982; Jackson, 1996a). The complex, yet slow pace of *Portia* behaviour raises questions about the potential limitation of cognitive capabilities in tiny brains.

The AL eyes of salticids are effective at detecting moving objects and can mediate orientation turns (Land, 1971; Duelli, 1978; Zurek et al., 2010), but their role has been poorly studied. There is evidence that the AL eyes play a role in quickly categorising moving objects and in the initiation of appropriate responses such as prey capture, even when the principal eyes are occluded (Forster, 1979; Zurek et al., 2010). Zurek and colleagues (2010) covered all eyes except the AL eyes in *Servea vestita* (now *S. incana*) and measured orienting responses to dot stimuli that varied in size and contrast, and that moved at different speeds. They found that all stimulus parameters had significant effects on orientation propensity. When tethered flies were used as prey, they found that visual information from the AL eyes alone was sufficient to elicit stalking behaviour (Zurek et al., 2010). These results suggest that the AL eyes support some degree of spatial acuity.

Salticids respond more readily to visual stimuli depicting prey than stimuli depicting non-prey, even when the stimuli are not real (e.g., digital projections Forster, 1985; Bednarski et al., 2012; Dolev and Nelson, 2014). Many salticids rapidly categorise objects as prey or non-prey based on only a few key features (Drees, 1952; Forster, 1985; Bednarski et al., 2012; Spano et al., 2012). An extreme example is with the salticid *Evarcha culicivora* which feeds indirectly on vertebrate blood by preferentially preying upon blood-fed *Anopheles* mosquitoes (Jackson et al., 2005; Nelson and Jackson, 2006). *E. culicivora* uses the resting posture and engorged abdomen characteristics of recently-fed *Anopheles* for identification (Nelson and Jackson, 2006; 2012). Dolev and Nelson (2014) investigated the predatory behaviour of *E. culicivora* toward different digital stimuli including abstract 'stick figure' representations of *Anopheles* constructed solely by known key identification elements, disarranged versions of these, as well as non-prey items and detailed images of alternative (non-preferred) prey. Spiders 'perceived' the abstract stick figures of *Anopheles* specifically as their preferred prey, attacking them significantly more often than non-

preferred prey, even when the comprising elements of the *Anopheles* stick figures were disarranged and disconnected from each other. However, if the relative angles between the elements of the disconnected stick figures of *Anopheles* were altered, the otherwise identical set of elements was no longer perceived as prey. These data showed that *E. culicivora* can make discriminations between prey items based on abstract concepts (Dolev and Nelson, 2014).

Previous studies investigating vision in the secondary eyes of salticids (Zurek et al., 2010; Zurek and Nelson., 2012bb; 2012aa; Melrose, 2015) have used stimuli that are not biologically relevant, such as a moving circle. Here, I aimed to determine the effect biological relevance (e.g., a prey item such as a fly) has on the response decrement to repetitive visual stimuli in the jumping spider, *Trite planiceps*. While any moving stimulus of the correct size is attractive to the salticid visual system (Forster, 1979; Zurek et al., 2010), I considered a moving circle as a biologically irrelevant stimulus and an image of prey (a fly) as a biologically relevant stimulus. Since salticids can discriminate features with the AL eyes alone (Forster, 1979; Zurek et al., 2010), I predicted that *T. planiceps* would remain responsive to the fly stimulus for longer (i.e., show a slower response decrement) than to the circle.

Methods

Methods, maintenance and feeding of spiders were as described in Chapter 2, apart from specific details outlined below. Both paired and unpaired tests were performed, all of which comprised 80 trials for each stimulus type presented. All experiments had an inter-stimulus interval (ISI) of 10 s and stimuli were presented against a light grey (RGB 250, 250, 250) background. Stimulus presentation was controlled using a custom written program using C#.NET on Microsoft Visual Studios© 2010. This program enabled control of stimulus type, size, vertical and horizontal location, velocity, duration, and ISI duration. Stimuli were created using Adobe Photoshop© CS5. Stimuli were designed to cross on the horizon of the salticid's field of view (i.e., 0^{o} vertically) and moved from posterior to anterior.

In the paired test, salticids (N=20) were shown a stimulus of a circle or fly across two monitors in a random manner such that each individual saw a mixture of both stimuli across 80 trials in one test. The stimuli used were a 40-pixel black circle or a 60-pixel fly. Because of its shape, the fly image contained white areas within the overall 40-pixel dimension, so it was set to 60 pixels to approximately match the size and amount of black space of the circle (black area was 26% and 17.6% for the circle and fly, respectively). For the unpaired tests, two experiments involving

different groups of spiders were run. In one, salticids (N=15) were shown a 40-pixel black fly presented across left and right monitors over 80 trials for each test, while in the other, salticids (N=20) were shown a 40-pixel black circle presented across left and right monitors.

Salticids were tethered to the apparatus as explained in Chapter 2. After being suspended in front of the apparatus, the salticid was given 20 min to settle from handling stress before being presented with the stimuli. Responses were scored as 5 different categories, where orientation toward the stimulus (causing the polystyrene ball held by the salticid to rapidly swivel in the opposite direction) was recorded as 1 or 2 (depending on which screen the stimulus appeared), fast responses were recorded as 3 or 4, general movement was scored as 5 and no movement or response as 0.

Responses were recorded and analysed as described in Chapter 2. Briefly, data were analysed in the software program R© (2016) with a cumulative link mixed-effect model which considered individual spider differences (i.e., intercepts). I used model selection, based on the Akaike Information Criterion (AIC), to determine an appropriate fixed-effects structure (containing the first term and second order interactions). The proportional odds model predicted the probability that a spider would fall into a specific response category across 80 trials. For visualisation, data were fitted in MatLab version 2015a with an exponential function $f(t) = \alpha e^{\lambda t}$ over all trials. Here, λ is the decay rate, α is the initial quantity of the response, and t is the current trial. For this, I omitted the 'no movement' category (category 0 in Chapter 2) and consequently plotted the exponential function of 'optomotor' (response category 3 in Chapter 2), 'fast' (response category 2 in Chapter 2), and 'activity' (response category 1 in Chapter 2) toward the stimulus in order to disambiguate any decrement in response toward the stimulus (fast and optomotor responses) from fatigue (general activity).

Results

In paired tests, salticids showed a decline in responses to both biologically relevant and irrelevant stimuli across trials (Fig. 3.1), showing only a 5% probability that *T. planiceps* remained responsive at the end of trials, transformed from estimates predicted by the model (Table 3.1). The decay rates shown in Fig. 3.1 for each response category were: 1. Optomotor response, circle = -0.1728 (CI = -0.2067, -0.1389), fly = -0.2120 (CI = 0.2512, -0.1727). 2. Fast response, circle = -0.0099 (CI = -0.0150, -0.0048), fly = -0.0121 (CI = -0.0173, -0.0069). 3. General activity, circle = 0.0079 (CI = 0.0016, 0.0142), fly = 0.0071 (CI = 0.0017, 0.0124).

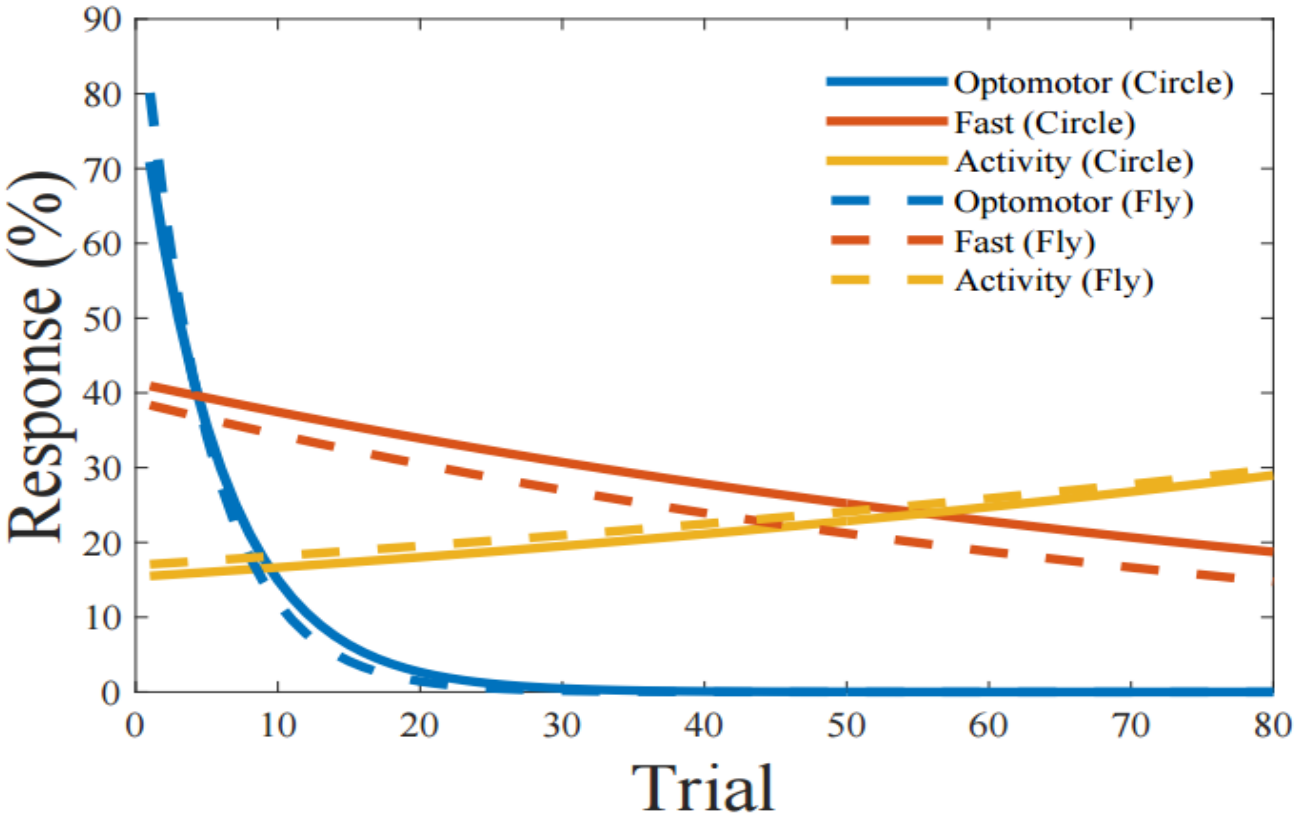

Figure 3.1. Decay rate (fitted as an exponential function over 80 trials) of the responses toward fly and circle stimuli in paired testing of *Trite planiceps* (N=20). Note: the 'no movement' category was omitted in the analysis to fit the exponential function. The yellow lines depict general activity, while the blue and red lines represent distinct responses toward the stimulus.

Model selection based on Akaike's Information Criterion (AIC) revealed that the model including trial only (excluding the stimulus type and presentation side) as a fixed effect was the best fit (trial only: AIC=3344.36, df=7; including stimulus type AIC = 3344.60, df=9). This outcome suggests that stimulus type and the side (left or right monitor) they were presented on did not have a significant effect on responses in *T. planiceps* (Table 3.2), but that responses decayed significantly over time. From the model selection results in Table 3.2 (and Melrose, 2015) it was concluded that

there was no monitor side bias, so I stopped recording stimulus presentation side for all experiments thereafter.

Table 3.1. Coefficient outputs from the cumulative link mixed effect model for repetitive biologically relevant and irrelevant visual stimuli on responses in *Trite planiceps*. Effect is the stimuli presented, estimates are those predicted by the selected model, transformation: % is the calculation taking the coefficient of the estimate through the R© package 'plogis', % is the transformed estimate shown as a percentage, where an increasing percentage represents decreasing rate of activity. N = 20 (fly vs. circle), 15 (fly), 20 (circle).

Effect	**Estimate**	**Transformation: %**	**Std. error**	**z value**	**P value**
Trial (fly vs. circle)	0.037150	0.951294: 95%	0.007594	4.892	9.99e-07
Fly at trial 1	-1.662694	0.1660886: 16%	0.324322	-5.127	2.95e-07
Circle at trial 1	1.662685	0.843817: 84%	0.324323	5.127	2.95e-07
Fly across trials	0.024863	0.9058804: 91%	0.008026	3.098	0.00195
Circle across trials	-0.024863	0.973421: 97%	0.008026	-3.098	0.00195

Table 3.2. Model selection results for evaluating the side of stimulus presentation (left or right monitor) on responses in *Trite planiceps*. Effects included are the fixed effects of interest and their pairwise interactions. K is the number of estimated parameters for each model, AICc is Akaike's Information Criterion, Δ AICc is the appropriate delta AIC component. AICcWt is the Akaike weights. N=20.

Effects included	**Model**	**K**	**AICc**	**Δ AICc**	**AICcW**
Trial only	T	7	3344.36	0.00	0.48
Trial and stimulus type information	(T+P)^2	9	3344.60	0.24	0.42
Trial and stimulus side information	(T+S)^2	9	3348.06	3.71	0.07
Trial, stimulus type and stimulus side	(T+S+P)^2	12	3350.03	5.67	0.03

There were three main effects of interest: T = trial, S = stimulus side, P = stimulus type
^2 indicates a pairwise interaction between effects

Raw data from the two independent tests were merged into the same file for statistical comparison. It was assumed that nothing between the time of testing for experiments 2 and 3 occurred that would affect responses, as spiders were kept in a constant laboratory environment and testing protocol were almost identical. Model selection based on AIC (Table 3.3) revealed that the model including the stimulus type as a fixed effect was the best fit, (including stimulus type: AIC=6449.07 df=9; excluding stimulus type AIC = 6467.54 df=7). This outcome suggests there was a significant difference in responses between the two experiments or stimulus types.

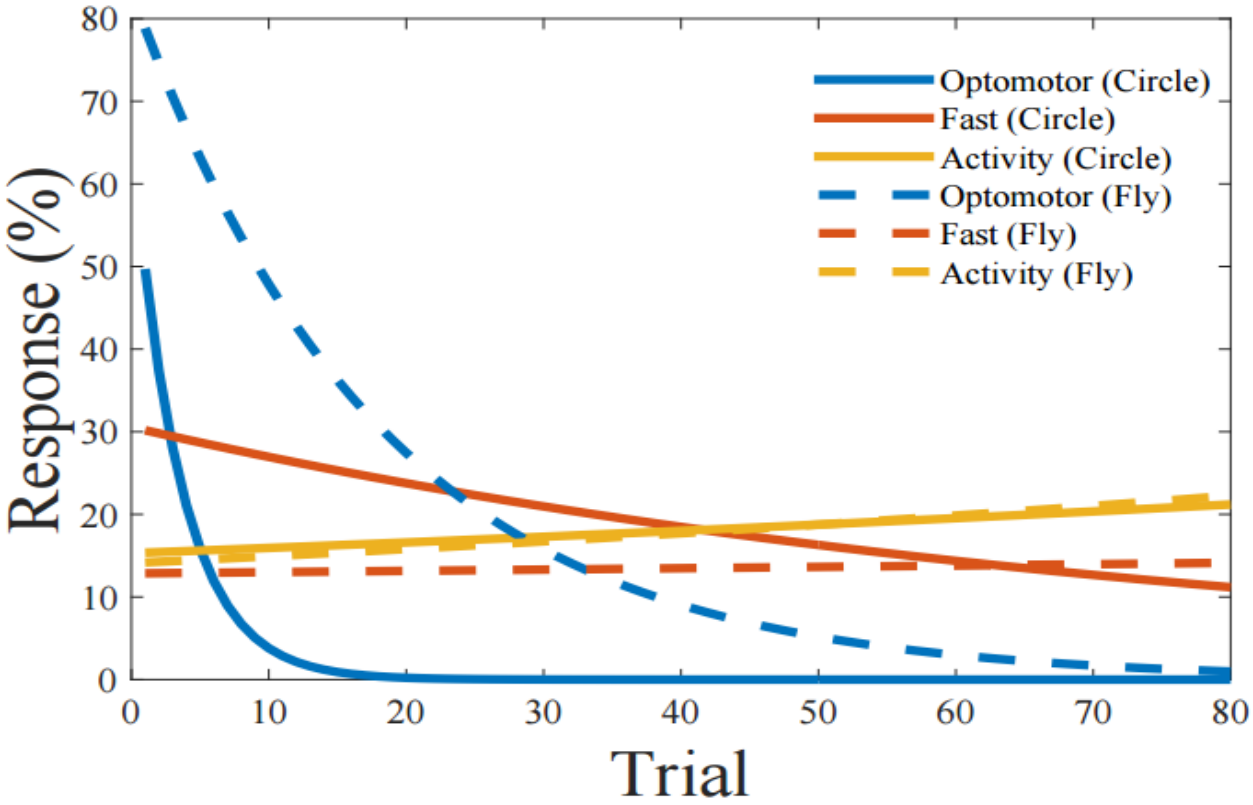

Figure 3.2. The decay rate of the responses in *Trite planiceps* fitted as an exponential function over 80 trials. One group of *T. planiceps* was shown a repetitive visual stimulus of a 40-pixel fly against a grey background, another group were shown a 40-pixel circle. Note: the 'no movement' category was omitted in the analysis to fit the exponential function. The yellow lines depict general movement, while the blue and red lines represent distinct responses toward the stimulus.

At the beginning of the tests (trial 1) salticids were much more likely to respond in a higher category to the fly stimulus (84%) than to the circle stimulus (16%) as transformed from estimates predicted by the model (Table 3.1). Across the 80 trials, *T. planiceps* showed a 9% chance of remaining responsive at the end of trials in the fly experiment and a 3% chance in the circle experiment (steeper decrement). The decay rates as shown in Fig. 3.2 for each response categories were: 1. Optomotor response, circle = -0.2858 (CI = -0.3379, -0.2337), fly = -0.0555 (CI = -0.0632, -0.0479). 2. Fast response, circle = -0.0126 (CI = -0.0166, -0.0085), fly = 0.0012 (CI = -0.0053, 0.0077). 3. General activity, circle = 0.0041 (CI = 0.0001, 0.0081), fly = 0.0057 (CI = 0.0011, 0.0103).

Table 3.3. Model-selection results for evaluating the stimulus type (fly or circle) on responses in *Trite planiceps* (N=35). Effects included are the fixed effects of interest and their pairwise interactions (unless otherwise stated). K is the number of estimated parameters for each model, AICc is Akaikes Information Criterion, Δ AICc is the appropriate delta AIC component. AICcWt is the Akaike weights.

Effects included	**Model**	**K**	**AICc**	**Δ AICc**	**AICcW**
Stimulus type	(T+P)^2	9	6449.073	0.00	1
Trial only	T	7	6467.539	18.44	0

There were two main effects of interest: T = trial and P = stimulus type
^2 indicates a pairwise interaction between effects

Discussion

In this study, *Trite planiceps* salticids were tested with biologically relevant (fly) and irrelevant (circle) stimuli. All experiments revealed a significant response decrement toward the stimuli. However, in the paired tests, response decay was similar for both the fly and the circle, whereas in the independent tests, the rate of decay for the fly was significantly slower than for the circle. The results from the unpaired tests support the hypothesis that the biological relevance of stimuli affects the response decrement in *T. planiceps,* particularly regarding optomotor responses.

The difference in the results from paired and unpaired tests is somewhat surprising. A possible explanation for this may lie in the nature of the images used as stimuli. The fly image included white space within the overall 40-pixel dimension, so it was set to 60 pixels in an attempt to match the size of the circle (see Appendix 2). Despite this, the area of black in the images still differed (circle: 1234 pixels of 4745 pixels = 26%, fly 1322 pixels of 7524 pixels = 17.6%). It is possible that the circle was perceived as a larger approaching object, which would explain the high responsiveness to it. The circle image also presents a higher contrast against the grey background than the fly image. Previous work (Zurek and Nelson., 2012a) has shown that while low contrast stimuli elicit orientation responses in salticids, there is a 'sweet spot' when it comes to the level of contrast and responsiveness in salticids. Deciding how much to enlarge the fly image to present an image of similar darkness as the solid circle was difficult. An even larger fly stimulus than the one I used may also have affected responses. In future, to replicate this experiment it would be better to replace the circle with a jumbled fly image (e.g., Tanaka et al., 1993; Tsao et al., 2003; Sheehan and Tibbetts, 2011) which preserves the same contrast and luminance of the fly image, while not resembling a fly.

The decrement observed in these experiments could be due to simple habituation, which would be characteristic of peripheral nervous system control, or it could be due to some sort of neural processing and under central nervous system control. If it were habituation I would expect the slopes to be very similar, which they were in the paired experiment. However, the results from the unpaired tests confirm that the response decrement is altered by the biological relevance of the stimuli. This is not unexpected because in *T. planiceps'* natural habitat it would be more beneficial to spend energy paying attention to potential prey than to general movement (e.g., leaves moving in the wind; Prete, 2004), which the circle may have represented. The observation that *T. planiceps* showed different response decrement to the two stimuli may suggest that the behaviour involves some sort of processing and is under central nervous system control because it appears that *T. planiceps* was making a choice about its responses to stimuli.

Aguilar-Argüello and García-Chávez (2015) investigated whether predatory strategies in the salticid *Corythalia albicincta* change with different prey types. These authors measured capture jump distance, time spent within each predatory stage (of the attach sequence) and attack efficiency in terms of prey capture*. C*. *albicincta* adjusted its behaviour in response to prey type; jump distance was greater with prey that could more readily escape (crickets and flies), compared to fly larvae. The duration of both latency and orientation was greater in larval trials than in the cricket and fly trials and *C. albicincta* spent less time in a crouching position with flies. The total duration of the predation process was shorter with flies than with crickets or larvae. Similar results have been described for the salticids *Yllenus arenarius* (Bartos and Minias, 2016) and *Evarcha culicivora* (Nelson et al., 2005) demonstrating the importance of prey type on predatory behaviour in salticids. These classifications are considerably more complex than those described for some vertebrates. For example, fiddler crabs*, Uca pugilator,* categorise stimuli as dangerous or harmless based on the position relative to the crabs' visual horizon, not based on the geographical horizon itself (Layne et al., 1997; Zeil and Hemmi, 2006). Layne et al. (1997) found that stimuli presented below the fiddler crab's horizon hardly ever evoked escape responses, whereas identical stimuli partially or entirely above the crab's horizon produced escape responses. The snapping behaviour of salamanders of the genus *Plethodon* has also been investigated. Roth (1987) tested a variety of plethodontid salamanders by presenting them with pieces of blackboard that differed in size and found a preference for snapping at smaller stimuli (2 or 5 mm). Earlier studies on natural diets in *Plethodon* salamanders have shown that the size of prey ranged from 0.5 to 7 mm in length in approximately 90% of stomach contents (Jaeger, 1972; Fraser, 1976). In contrast, identification of

'head-like' cues and their alignment with direction of movement may be a fundamental aspect of salticid predatory behaviour, as suggested from studies by Bartos and colleagues (Bartos, 2007; Bartos and Minias, 2016) and by Moss and colleagues on wax secreting aphids and their recognition as prey by *Marpissa marina* (Moss et al., 2006b)

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Chapter 4

Does caffeine effect how jumping spiders behave?

Introduction

Behaviours that are controlled by the peripheral nervous system (PNS), such as sensory habituation, are typically less complex than behaviours under central nervous system (CNS) control (Bernardo et al., 1977). The crayfish, *Procambarus clarkia*, exhibits a typical example of habituation. If a tactile stimulus is applied to the abdomen or tail fin of a crayfish, it will respond by somersaulting upwards and forwards, away from the stimulus. This is caused by rapid flexion of the abdominal musculature (Araki and Nagayama, 2005; Nagayama and Araki, 2015). The lateral giant interneuron receives combined information from the primary afferent (peripheral) neurons and sensory interneurons in the abdomen. These trigger excitatory synapses with giant motor neurons which stimulate the fast flexor muscles in the respective body segments of the animal, propelling it through the water in a classic reflex response (Mittenthal and Wine, 1973). If a tactile stimulus is repeatedly applied, however, the tail fin escape behaviour diminishes and the animal habituates by showing a response decrement. It has been shown that, when extracted from the crayfish, the lateral giant interneuron mediating the behaviour habituates by reducing its excitability (Nagayama and Araki, 2015). Response decrement through habituation to an irrelevant stimulus is a widespread phenomenon, having also been demonstrated in the 'simplest' of organisms, including the unicellular ciliate *Stentor* (Wood, 1988) and in slime moulds (Boisseau et al., 2016; Vogel and Dussutour, 2016).

Neuronal responses to repeated stimuli often show a fast decline, either in the form of habituation, or through more complex associative learning processes (Sztarker and Tomsic, 2011; Berón de Astrada et al., 2013; Liedtke and Schneider, 2014). Human vigilance studies show that after some time, we also show a response decrement (or 'vigilance decrement') to a repeated stimulus. Vigilance is the ability of organisms to remain attentive to stimuli over extended periods (Parasuraman, 1976; Davies and Parasuraman, 1982), but it does decline over time. When carrying out a prolonged mundane task, such as searching for a subtle target (e.g., the letter 'D') amongst a series of distractors (e.g., the letter 'O'), participants are less likely to detect targets, and therefore

respond less, towards the end of a 20 min period (Mackworth, 1968; Davies and Parasuraman, 1982; Warm, 1993; Temple et al., 2000; Helton and Warm, 2008; Pattyn et al., 2008). The difference between response decrement in vigilance studies and those observed in the crayfish and 'simpler' animals may reside in the underlying causal mechanisms. In crayfish habituation, the behaviour is governed through the PNS. In human vigilance studies, the observed response decrement is thought to be more complex and primarily driven by the CNS. The participants can still see the stimulus (i.e., it is not filtered out by the sensory system), but they do not respond to it. This may occur either through 'boredom' (Pattyn et al., 2008) or cognitive fatigue (Helton and Warm, 2008). The difference between these concepts is that the former seeks to explain response decrement due to the lack of cognitive loading (cognitive understimulation), and the latter posits that the decrement is a result of cognitive overstimulation.

Drugs can help distinguish if a behaviour is under CNS or PNS control (Witt and Reed, 1968). They affect invertebrates analogously to how they affect vertebrates (Christiansen et al., 1962; Witt, 1962; Witt and Reed, 1965; Witt, 1969; Witt, 1971; Barron et al., 2009), despite differences in the nervous systems between the two. Witt and colleagues (1949; 1962; Witt and Reed, 1968; 1971) and others (Mardikar et al., 1969; Hesselberg and Vollrath, 2004) have investigated the effect of drugs on web-building in spiders. Drug studies in spiders began in 1948 when Dr Hans Peters was studying web building behaviour in house spiders, *Araneus diadematus*. It was thought that with stimulant drugs, web-building could be shifted (forward) to a later hour (as cited by Witt et al., 1968b). Because web-building is a CNS-controlled behaviour (Hesselberg and Vollrath, 2004; Eberhard, 2011), drugs that alter CNS functions, such as amphetamines, were investigated. Instead of building normal webs at a later hour, spiders built badly distorted webs at the usual time, suggesting that the drugs were interfering with an otherwise consistent CNS-controlled behaviour. Across many studies, house spiders, *A. diadematus*, *Araneus sericatus,* and other species have been exposed to a variety of drugs (Witt and Reed, 1968; Mardikar et al., 1969). Spiders given cannabis build webs normally but stopped three-quarters of the way through (Noever et al., 1995), while those dosed with benzedrine ('speed') spun vigorously, but erratically. Spiders given caffeine produce highly distorted webs (Witt and Reed, 1968; Noever et al., 1995; Hesselberg and Vollrath, 2004) and chloral hydrate, an ingredient in sleeping pills, made the spiders inert after barely getting started on the web (Noever et al., 1995). By comparing behaviour from a drugged state with that found in a normal state, this knowledge can be employed to test if a behaviour is under CNS or PNS

control. If a behaviour is measured when an animal has been administered a CNS stimulant and, in a paired manner is also tested with a non-drug control such as water, the behaviour between treatments should not change if it is under PNS control. However, if the behaviour changes when animals have taken a CNS stimulant, this suggests that the behaviour in question is under CNS control.

Jumping spiders (Salticidae) are comparatively small spiders (2-10 mm as adults), with short legs and a broad, square cephalothorax that bears strikingly large eyes. They typically do not use webs to catch prey, and instead use silk to build nests in which to lay eggs and rest (Foelix, 2011). Salticids are persistent cat-like solitary hunters and are very attentive to moving stimuli; after a quick turn to face an object, they may move closer, and in the case of potential prey, slowly stalk it and pounce (Foelix, 2011). Salticids observe their environment using a combination of two types of eyes. The forward-facing pair of 'principal' eyes have narrow fields of view, but combine excellent spatial resolution with colour vision, while the three pairs of 'secondary' eyes have wide fields of view and function primarily as motion detectors (Duelli, 1978; Zurek and Nelson., 2012a). The largest pair of secondary eyes, the anterior lateral (AL) eyes, is also forward-facing, while the two remaining pairs are found along the side of the cephalothorax. Principal and secondary eyes have a combined field of view extending almost 360 degrees (Land, 1985). Motion detected by the secondary eyes may elicit an orienting response, whereupon the object of interest is examined further using the high-acuity principal eyes.

In the experiment described in this chapter I used randomised paired testing to investigate the effect of caffeine (a CNS stimulant) on the orienting response decrement toward a repeated visual stimulus presented to the AL eyes of the salticid *Trite planiceps*. I predicted that *T. planiceps* would show a more gradual response decrement after they had been given caffeine compared to having received water (control) on the assumption that the response decrement observed in this species (Melrose, 2015; Chapters 2 and 3) is under CNS control.

Methods

Methods, maintenance, feeding of spiders and testing protocol were as described in Chapter 2, with alterations outlined below. Preliminary tests were conducted to determine the best method of drug administration and dosage. To reduce the number of confounding variables, administration of sugar within the caffeine solution and depriving animals of water prior to experimentation were avoided. These tactics were typical in previous experiments (Witt, 1971; Samu and Vollrath, 1992;

Hesselberg and Vollrath, 2004), but it was apparent that the salticids drank the solution without the need to be thirsty or for added sweetness.

Salticids were randomly assigned either to a 'water first' or a 'caffeine first' condition and tests were conducted when they had been without food for 6-8 days. Each salticid was tested twice; once with caffeine and once with distilled water. For caffeine tests, 75 mg of crystalline caffeine (trimethylxanthine) was dissolved in 25 ml of distilled water. Test salticids (N = 15) were weighed before dosing so that the amount of solution administered could be weight-adjusted such that each animal received c. 26 µg of caffeine per 1 mg of body weight (Hesselberg and Vollrath, 2004). Individuals were placed inside the 'gunkatron': a cylinder that was open at one end, with holes drilled in the opposite, closed, end. Another cylinder, capped with foam onto which a spider was placed, was sized such that when it was plunged into the first cylinder, the spider could be immobilised without the need for carbon dioxide and its cephalothorax could be accessed through the holes in the outer cylinder. Salticids were positioned within the gunkatron with their anterior side facing up so that the mouthparts could be accessed. Once in position, an Eppendorf Varipette® 4710 was used to apply a small droplet (mean droplet volume 4.48 (± 0.22 SEM) µL, n = 30), either of water or of caffeinated water, to the mouthparts, which they would readily ingest within 30 min. To ensure that the salticids were ingesting the solution, a paper towel was placed underneath their body to expose any solution trickling past their mouthparts (which, in fact, never occurred).

Once the solution was ingested, salticids were tethered to the apparatus, as described in Chapter 2. After being suspended in front of the apparatus, the salticid was given 20 min to settle from handling stress and to allow time to digest the solution, before being presented with a stimulus movie. The stimulus movie consisted of a low contrast 'signal' (circle) of 10% opacity (RGB 239, 239, 239) moving across a moderately 'noisy' screen (clutter level 2) and was created in Adobe Premiere®, as described in Chapter 2, so that the circle appeared behind clutter.

Preliminary data had already established that there is no bias for left or right screens (see Chapter 3; Melrose, 2015). Responses over 80 trials were recorded and analysed as described in Chapter 2. Briefly, data were analysed in the software program R© (2016) with a cumulative link mixed-effect model which considered individual spider differences (i.e., intercepts). I used model selection, based on the Akaike Information Criterion (AIC), to determine an appropriate fixed-effects structure (containing the first term and second order interactions). The proportional odds model predicted the probability that a spider would fall into a specific response category across 80

trials. For visualisation, data were fitted with an exponential function $f(t) = \alpha e^{\lambda t}$ over 80 trials. Here, λ is the decay rate, α is the initial quantity of the response, and t is the current trial. For this, I omitted the 'no movement' category (category 0 in Chapter 2) and merged the 'fast response' and the 'very fast response' (categories 2 and 3 in Chapter 2). Consquently, I plotted the exponential function of 'activity' (response category 1 in Chpater 2) and of 'interest' (response) toward the stimulus in order to disambiguate any decrement in response toward the stimulus from general fatigue.

Results

Model selection revealed that the model including 'treatment' (caffeine or water) as a fixed effect was the best fit (including caffeine: AIC=4328.12, df=9; excluding caffeine AIC = 4553.56, df=7), showing that caffeine had a significant effect on responses in *T. planiceps* in such a way that the spiders maintained their responses to the stimulus for longer (Table 4.1; Fig. 4.1).

Table 4.1. Model-selection results for the significance of treatment (caffeine or water) on responses to a repetitive visual stimulus in *Trite planiceps* (N=15). Effects included are the fixed effects of interest and their pairwise interactions. K is the number of estimated parameters for each model, AICc is Akaikes Information Criterion, Δ AICc is the appropriate delta AIC component. AICcWt is the Akaike weights.

Effects included	**Model**	**K**	**AICc**	**Δ AICc**	**AICcW**
Caffeine treatment	(T+C)^2	9	4328.12	0.00	1
Trial only	T	7	4553.56	225.43	0

There were two effects of interest: T = trial and C = caffeine treatment
^2 indicates a pairwise interaction between effects

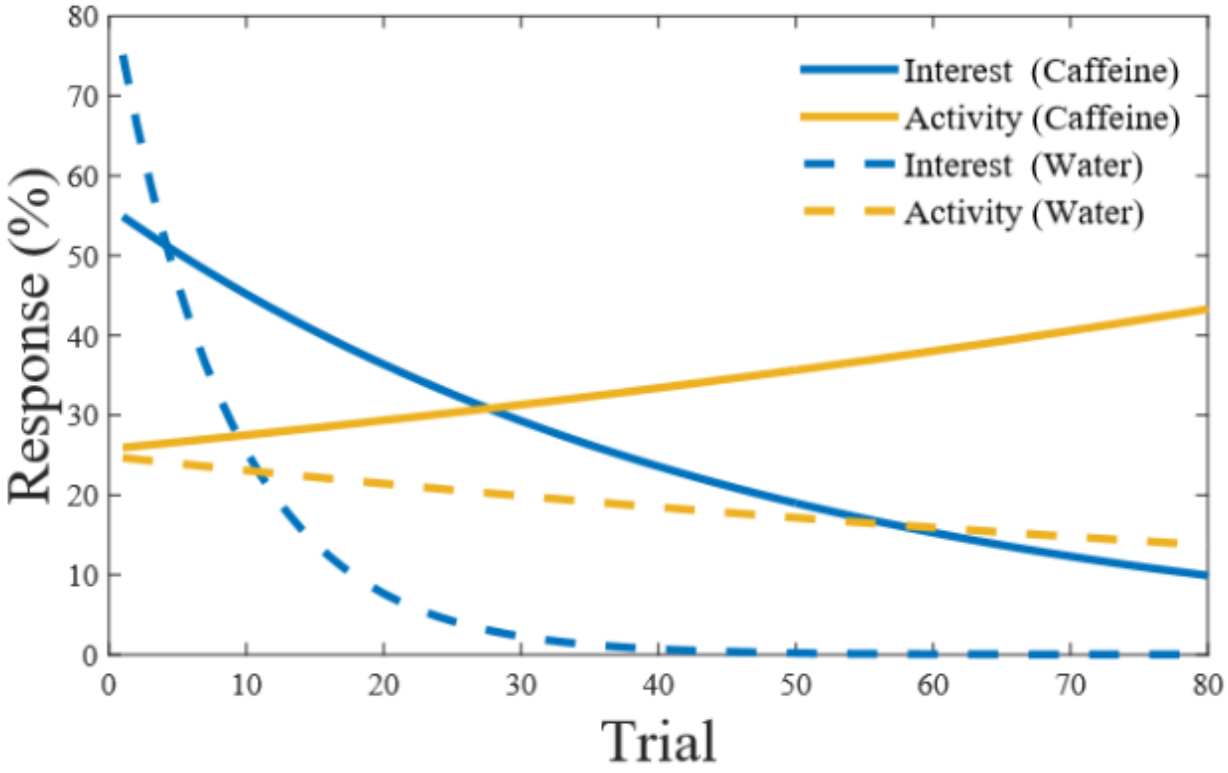

Figure 4.1. Decay rate of the responses in *Trite planiceps* fitted as an exponential function over 80 trials. Spiders received a caffeine droplet and distilled water droplet before being shown a repetitive visual stimulus of a circle of 10% contrast amongst a low-medium level of clutter. The yellow lines depict general movement, while the blue lines represent distinct responses toward the stimulus.

In both treatments, there was only a 10% chance that *T. planiceps* remained responsive at the end of trials (Table 4.2, row 1). At the beginning of the tests (trial 1), *T. planiceps* was much more likely to respond in an 'interest' response category when given caffeine (72% chance) compared to when given water (28% chance, Table 4.2). Across time, when *T. planiceps* was given caffeine, there was a higher chance that they remained responsive at the end of trials compared to when they were given water (Table 4.2, Fig. 4.1), revealing a significantly slower response decrement when they were given the CNS stimulant. Decay rates for the slopes depicted in Fig. 4.1 for each response category are: interest; caffeine=-0.0217 (CI=-0.0273, -0.0161) water=-0.1202 (CI=-0.1581, -0.0823) activity; caffeine=0.0065 (CI=0.0026, 0.0103) water=-0.0074 (CI=-0.0128, -0.002). In both treatments, salticids showed a decline in responses to the repetitive visual stimuli across the 80 trials, but the decrement in response was much steeper in the control treatment (Fig. 4.1). The observed decrement in response to the stimulus was not paralleled by general movement so was not due to fatigue. Indeed, spiders in the caffeine treatment increased their general activity over time (Fig. 4.1).

Table 4.2. Coefficient outputs from the cumulative link mixed-effect model for repetitive visual stimuli presentation on responses in *Trite planiceps* that were given caffeine and water (control). Effect is variable of interest, estimates are those predicted by the selected model compared to control treatment, transformation: % shows the coefficient of the estimate through the R© package 'plogis' with % being the transformed estimate shown as a percentage (an increasing percentage represents decreasing rate of activity).

Effect	Estimate	Transformation: %	Std. error	z value	P value
Trial	0.02697	0.8964: 90%	0.004831	5.584	2.34e-08
Caffeine treatment at trial 1	-0.96603	0.2793: 28%	0.175407	-5.507	3.64e-08
Trial and caffeine treatment	-0.00865	0.6224: 62%	0.003958	-2.188	0.0287
Water treatment at trial 1	0.96603	0.7297: 73%	0.175406	5.507	3.64e-08
Trial and water treatment	0.00866	0.9579: 96%	0.003958	2.188	0.0287

Discussion

This study was the first to test the effect of caffeine, or any drug, in salticid spiders. Here salticids were given a drop of distilled water to drink, and in a paired treatment they received caffeinated distilled water and responses to repetitive visual stimuli were subsequently measured. This experiment revealed a significant difference in the decay of responses. When spiders received a caffeine droplet they responded to the stimulus for much longer (slower decrement) than they did when given water. I ruled out fatigue as a cause for the observed response decrement because spiders remained actively moving throughout both tests. In fact, in the caffeine treatment, general activity levels increased over time, suggesting that caffeine was producing a stimulant effect on the spiders. This result supports the hypothesis that the response decrement observed in *T. planiceps* (see Chapters 2 and 3) is centrally regulated. It also suggests their behaviour is not due to sensory habituation, as the prediction based on that hypothesis was that there would be no difference between treatments. Consequently, the response decrement behaviour observed in salticids may be considerably more complex than it initially appears, despite their relatively simple nervous systems (Foelix, 2011).

Simple sensory habituation is controlled by the PNS and is the most basic form of learning. Current thought typically places CNS-based behaviour as more 'reflective' rather than 'reflexive', and consequently is believed to be more complex (Pearce, 1997; Barron et al., 2015). The simplicity of habituation is evident in that the lateral giant axons in crayfish neurons (outside of the animal) and unicellular organisms both display habituation (*Stentor*; Wood, 1988; and slime mould Boisseau

et al., 2016; Vogel and Dussutour, 2016). Studies in other animals have shown that response decrement is PNS-regulated (Geer, 1966; Johnson and Wuensch, 1994; Deecke et al., 2002; Nagayama and Araki, 2015). Nordström et al. (2013) found that visually-guided behaviours in the crab *Neohelice granulata*, such as ignoring a repetitive visual 'danger' stimulus, is determined by neural plasticity that occurs early in the visual pathway. Interneurons in locusts also show a PNS-regulated behavioural response (Gray, 2005). The experiment described here has demonstrated that what appears to be habituation in salticids is actually more complex, being centrally-mediated. Indeed, it could be argued that the behaviour observed in *T. planiceps* is better described as a vigilance decrement, terminology commonly used in human studies (e.g., Mackworth, 1968; Warm, 1993; Temple et al., 2000; Helton and Warm, 2008).

Previous drug studies in spiders involved orb weavers, such as the house spider *Araneus diadematus*. In the web-building studies, caffeine-affected spiders spun the most distorted webs, even when compared to amphetamines or LSD (Witt and Reed, 1968; Witt, 1971). The reason behind this remains unclear, but it could be due to the doses of caffeine used. In those studies, spiders received up to 100 µg of caffeine per individual (1 g/kg for a 100 mg spider), which severely handicapped many of them - indeed, it was surprising that they could construct webs at all (Witt, 1949; Witt and Reed, 1968). The amount of drug in the body determines the extent of the effect (i.e., there is a drug concentration relationship; Witt, 1971) and in later studies (Hesselberg and Vollrath, 2004), doses of approximately 26 µg per unit of body weight were used instead. Spiders under these doses produced rounder webs compared to controls; and, perhaps counterintuitively, at slower speeds (Witt, 1971; Hesselberg and Vollrath, 2004). Even though the differences here may not be as 'intuitive' as those found at higher doses due to the speed at which the webs were built at lower doses, it was apparent that, regardless of dosage, spiders given caffeine did not behave as they normally did. At high caffeine doses, they behaved extremely erratically, while at lower doses they appeared to behave abnormally 'carefully'. Similarly, the results described in this chapter showed clear effects of caffeine on the behaviour of the salticid *T. planiceps*.

Caffeine dosage elicits vastly different behavioural responses in several invertebrates (Mustard, 2014). In the jellyfish, *Aurelia aurita,* caffeine was given at very high concentrations and resulted in inhibition of swimming behaviour (Schwab, 1977). When *Drosophila* were exposed to caffeine before conditioning, their ability to associate a visual cue with an aversive stimulus was severely reduced (Folkers and Spatz, 1984; Wang et al., 1998). Mustard and colleagues (2014) tested honey

bees, *Apis mellifera*, with repetitive olfactory conditioning and showed that when the bees were fed an acute dose of caffeine in the reward solution (either prior to or during conditioning) they show a reduced level of acquisition. In contrast, many other studies suggest caffeine increases learning in vertebrates (Mustard, 2014). For example, honey bees, *Apis mellifera*, fed caffeine in a reward solution performed better during massed olfactory training than those fed sucrose alone (Wright et al., 2013). Additionally, in the common snail, *Helix lucorum,* acquisition of a conditioned response was increased after being injected with caffeine either before or after a conditioning trial (Silant'eva et al., 2009). It is thought that the difference in effects of caffeine in learning is due to the concentration used (Mustard, 2014). Experiments that resulted in a negative learning effect of caffeine (Folkers and Spatz, 1984; Mustard et al., 2012) used relatively high concentrations (10 – 15 Mm) whereas experiments that resulted in positive learning effects (Silant'eva et al., 2009; Wright et al., 2013) used lower concentrations (0.1 – 100 μM).

Very small species such as spiders display a level of behavioural complexity (e.g., intricate web building or detouring in *Portia;* (Tarsitano and Jackson, 1997) that is comparable to the behavioural complexity of larger animals (e.g., maze solving in rats and mice; Cahill et al., 2015), despite having less neurons than larger animals (Beutel et al., 2005). This raises the question of behavioural limitation in small species (Eberhard, 2007; 2011). Small species probably have less complete sensory information and processing due to having fewer sense organs and interneurons (Eberhard, 1990; 2000). The fact that they display complex behaviour despite a smaller CNS led to the 'size limitation hypothesis' that states that small species should suffer especially strong selection to reduce the cost of the CNS, which may make them more likely to sacrifice behavioural capability to economise on nervous tissue (Eberhard, 2011). Two extreme possibilities have been suggested: 1. small species have evolved sufficient adjustments in brain size and design that enable the same behavioural capacities as those of larger, related, species, or, 2. they have smaller brains leading to different behaviour (Eberhard, 2007; 2011). To explore these suggestions, Eberhard (2007; 2011) compared web construction behaviour of small orb-weaving spiders to those of relatives up to 10^4 times larger. He hypothesised that the if the behavioural limitation hypothesis is true, then to achieve a similar level of precision in web building, the behaviour of especially small spiders might be slower. Eberhard's results (2007; 2011) contradicted these expectations, and demonstrated that in web building (at least), behavioural precision and speed is not limited by size.

In orb weavers, different neurotoxins disturb different parts of the web-building programme, presumably through their action on the CNS (Hesselberg and Vollrath, 2004; Eberhard, 2011). For example, methamphetamine affected the size of the catching area in webs, and the regularity of angles and spirals, whereas spiders on atropine built webs of normal size and regularity but used wider meshes (Witt and Reed, 1968). Future research should further explore the effects of drugs in salticids. Because salticids display distinctly different hunting behaviour to orb weavers (stalking, prey choice, pouncing etc.), this could provide important insight into learning, decision making, and other cognitive abilities. Altering the types and doses of drugs administered could also provide insight into cognitive capabilities of salticids, which have been intensively studied due to their complexity (reviewed in Jackson and Cross, 2011). The results from this experiment demonstrate that the platform for this avenue of research is now established.

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Chapter 5
General discussion

Synopsis of experiments and main findings

My findings from this thesis strongly suggest that the response decrement observed in the jumping spider *Trite planiceps* is under central nervous system (CNS) control and is likely to be a cognitive behaviour. None of my results suggest that this is sensory habituation under peripheral nervous system (PNS) control. I will discuss the reasons for my overall conclusion in the same order as the chapters presented in this thesis; first, the effect of difficulty (Chapter 2), then biological relevance (Chapter 3) and finally the effect of a CNS stimulant, caffeine (Chapter 4).

In Chapter 2, I asked if task difficulty (in terms of stimulus salience and clutter intensity) would affect the response decrement in *T. planiceps*. I predicted that, if the response decrement was due to understimulation, the spiders would remain more responsive towards the end of trials in difficult tasks than in easy tasks, on the assumption that difficult tasks would be more 'engaging'. I found the opposite trend; *T. planiceps* was more responsive at the end of trials (i.e., showed a more gradual decrement) in tasks that were easier rather than in tasks that were difficult. I found a ceiling effect in the high clutter conditions, where it was apparent that the task was too difficult. Hunger did not factor as a big effect, suggesting that any difference in responses was not primarily due to motivation. In the harder tasks, responses at the beginning of tests were higher than in the easier tasks, presumably because it took more effort to disambiguate the circle in hard tasks. In the harder conditions, after the first few trials, responses tapered off quickly, probably because it was cognitively demanding to maintain the level of selective attention required for those tasks. Consequently, the response decrement observed in the experiment described in Chapter 2 was most likely driven by some sort of CNS overstimulation (or resource depletion theory, in human vigilance studies; Helton et al., 2005; Helton and Warm, 2008) as opposed to understimulation (Pattyn et al., 2008).

In Chapter 3, I investigated whether the biological relevance of stimuli would affect the response decrement in *T. planiceps*. In one experiment, there was no difference in responses to both stimulus types (circle versus fly), probably because of the nature of the images used for stimuli (see details in Chapter 3). In the other tests, considerably more 'urgent' optomotor responses were observed and the response decrement was less steep for the fly stimulus compared to the circle

stimulus. Thus, the biological relevance of stimuli did affect the response decrement in *T. planiceps*, perhaps suggesting some form of processing or decision making.

In Chapter 4, I considered whether a CNS stimulant, caffeine, would affect *T. planiceps*' response decrement based on the premise that if the observed behaviour was under CNS control, then administering a CNS stimulant would change the slope of response decay. Both 'interest' responses and general activity were higher when spiders were given caffeine, compared to when they were given water. Because this was a paired test, the observed increases are strong evidence that the response decrement observed in *T. planiceps* is under CNS control.

Interpretation

Previous work on the response decrement in *T. planiceps* suggested that this species of jumping spider habituated to repeated presentation of visual stimuli (Melrose, 2015). It was thought that because the behaviour was in accordance with certain habituation properties (e.g., smaller inter-stimulus interval resulted in more pronounced cessation of responses) that the response decrement was due to sensory habituation. However, the properties affecting the response decrement needed to be investigated further, which was the aim of this thesis. From personal observation, it became clear after a few tests that what I was observing in *T. planiceps* was not simply habituation. For example, it was rare to see complete cessation of responses. Almost all spiders would reduce or stop responding after 10-20 trials but would partially resume responding round trial 60 (something which is also apparent in the data in Melrose, 2015). In saying that, individual spiders were often consistent in their responses, and these responses varied markedly from spider to spider; some spiders were always inert after the first several trials, while others were always active and responsive throughout tests. If the phenomenon were habituation I would expect a complete cessation of responses (Barry, 2009; Rankin et al., 2009). Additionally, previous work in this species found no evidence of dishabituation (Melrose, 2015), which is considered an important indicator of sensory habituation processes.

Balderrama and Maldonado (1971) investigated habituation in praying mantises, *Stagmatoptera biocellata*, through measuring their fright response to repeated presentations of three different bird species. They found effects, which were retained for six days, for all three stimuli: a decrease in the number of fright responses, an increase in the percentage of incomplete responses, and a shortening in the response duration (Balderrama and Maldonado, 1971). In crabs, *Neohelice granulata*, the neural switch for adaptation to repeated visual information has been

found to take place at an early stage of the visual processing pathway and it is not due to neuronal or behavioural fatigue (Berón de Astrada et al., 2013; Nordström and Gonzalez-Bellido, 2013). These results suggest that the response decrement found in praying mantises and crabs is under PNS control (i.e., habituation). My results, however, suggest that the response decrement observed in *T. planiceps* most likely involves the CNS.

In the human vigilance literature there are two main theories explaining vigilance decrement; the understimulation or mindlessness theory (Pattyn et al., 2008) and the overstimulation or resource depletion theory (Helton and Warm, 2008). Support for both theories has been found. While less work has been done on vigilance decrement in non-human animals, there is some evidence for limited attention (i.e., overstimulation) in animals (Dukas and Kamil, 2000; 2001; Kaby and Lind, 2003), as also suggested by the results presented in Chapter 2. When animals are engaged in complex activities their ability to focus on potential threats is probably reduced and their anti-predatory actions may be delayed. For example, Dukas and Kamil (2000) investigated attention ability in blue jays, *Cyanocitta cristata*, using simulated foraging scenarios. The birds were trained to distinguish and peck at targets on a computer monitor. When a task was difficult (many distracting objects on the monitor) the birds lost some attentional capacity for peripheral targets. Kaby and Lind (2003) also investigated task difficulty (foraging) on attention in birds. The authors simulated raptor attacks to blue tits, *Parus caeruleus*, and measured predator detection and time to take-off when in different levels of foraging task difficulty; not foraging, foraging on chopped mealworms, or foraging on whole mealworms. When birds were foraging on chopped mealworms their detection compared to non-foraging birds was unimpaired. However, detection and time to take-off was delayed more than 150% when the birds were foraging on whole, live mealworms, which apparently demanded much attention and handling skill (Kaby and Lind, 2003). These studies suggest that the difficulty of the task (e.g., foraging or background complexity) is an important consideration when studying vigilance.

In addition to task difficulty, there is evidence that biological relevance increases response rate. The results from Chapter 3 also generally conform to this concept, and this may be centrally-mediated as the neural firing rate in the CNS of salticids has been found to be significantly greater in response to an image of an intact fly compared with an image of a scrambled fly (Menda et al., 2014). The biological relevance of stimuli appears to alter not only more complex responses, but also simple responses, such as habituation. Hemmi and Merkle (2009) tested the response of

fiddler crabs, *Uca vomens*, to repeatedly approaching dummy predators to find out whether the crabs would habituate to potential predators and to test how selective the habituation process may be. The authors found that crabs habituated to model predators, even though they were confronted with real predators during the same habituation process (Hemmi and Merkle, 2009).

Honeybees have recently been described and used as ideal models for testing the effect of addictive drugs on nervous systems (Menzel, 1999; Abramson et al., 2000; Menzel, 2001; Giurfa, 2007; Ammons and Hunt, 2008; Barron et al., 2009). Examples include: cocaine increasing the likelihood and rate of bees dancing after foraging (Barron et al., 2009); addictive drugs modulating learning and memory (Chen et al., 2014); ethanol ingestion impairing appetitive olfactory learning and odour discrimination (Mustard et al., 2008); caffeine reducing the age at which bees are able to learn in an olfactory associative task (Si et al., 2005); lastly, acute morphine injection impairs the acquisition of short- and long-term associative memory and disrupts consolidation of the long-term memory (Fu et al., 2013). Witt and others (Witt, 1949; Christiansen et al., 1962; Witt and Reed, 1965; 1968; Witt et al., 1968a; Witt, 1971) have shown many times that drugs affect a CNS-mediated behaviour, web building, in orb weavers, *Araneus diadematus.* In this thesis, salticids were tested with caffeine for the first time and proved to have the potential to become a model system for testing the effects of drugs on the nervous system, as, like honeybees (Menzel, 1999), salticids are well-known for their complex and cognitive behaviour (Harland and Jackson, 2004; Jackson and Cross, 2011). In Chapter 4, I found that *T. planiceps* willingly ingested an unsweetened caffeine solution within 30 min of receiving it and that this subsequently influenced their behaviour.

The combined results from the experiments described in this thesis show that response decrement observed in *T. planiceps* was stronger (faster) when task difficulty was high, possibly due to overstimulation of the CNS. Decrements were also faster with less biologically-relevant stimuli, and were significantly weaker (slower) when given a CNS stimulant. Taken in combination, these results strongly suggest that the response decrement is controlled by the CNS and that perhaps it is a cognitive process, possibly involving interest.

Unexpected findings and limitations

In all experiments described in this thesis (raw data in Appendices 1-3 and see; Melrose, 2015), the 'second peak phenomenon' was consistently observed. This is when salticids re-commenced responding in later trials (usually at about trial 60) after a period of low responses. There are at least two reasons that might explain the second peak: a renewable resource model, and

spontaneous recovery. The findings described in Chapter 2 reflect those found in resource depletion or overstimulation theories for vigilance decrement studies (Grier et al., 2003; Helton et al., 2005; Helton and Warm, 2008). Thus, it could make sense to speculate that the second peak observed in *T. planiceps* responses occurred after a 'micro break'. Here, perhaps the neural system balances effort with demand, and to avoid overtasking, switches attention off to allow neurons to 'replenish' (Helton et al., 2002). Alternatively, it could be due to spontaneous recovery, which is the re-emergence of a previously extinguished conditioned response after a delay (Benjamin, 2007). In this scenario, the spiders may have learned to stop responding, perhaps through lack of reward, but after numerous stimulus presentations (60 or so trials), they 'forgot' that they could not get the reward, and so started responding again, as has been suggested for rats (Bouton et al., 2004; Benjamin, 2007). This, however, seems unlikely as rewards were not implemented in my experiments and spiders were not conditioned. Additionally, almost no jump responses (where a salticid drops the polystyrene ball in an attempt to jump) were observed, which would presumably represent the salticids attempt to feed or obtain a reward.

Overall, the main limitations of this study were that the scoring of responses in *T. planiceps* was not done by a blind scorer, and this may have caused unintentional biases in the results. Additionally, a robotic tracking ball for salticids to hold instead of a polystyrene ball could be programmed to have a definite cut-off speed and degree of rotation to more accurately distinguish an optomotor response (swivel speed of c. 700^{o}/s) from a 'fast' response. This would eliminate observer bias and save time. For the experiments described in Chapters 2 and 4, stimuli were presented through a media player instead of the custom-built programme described in Chapter 3. Presenting all stimuli with the custom-made programme would ensure consistency. These limitations are relatively minor and I do not think they affect the validity of my findings.

Future directions

Future testing of biological relevance on response decrement could use images and olfactory properties of predators, prey and mates. For example, it would be interesting to test if responses to known predators of *T. planiceps* (e.g., the earwig *Forficula auricularia*, and spiders from other families, such as *Clubiona cambridgei, Cheiracanthium stratioticum* and *Zelanda erebus* (Vink et al., 2011) would elicit slower decay rates than toward non biologically relevant images (e.g., circle), as suggested by my results with potential prey in Chapter 3. Additionally, olfaction tests might also reveal differences in response decay, as it is known that salticids respond to chemosensory cues of

prey, predators, and conspecifics (Jackson et al., 2005; Nelson and Warui, 2012; Nelson and Jackson, 2014). For example, would hungry salticids remain responsive for longer in a circle stimulus experiment (comparable to the one in Chapter 2) when smells from prey were present? Responses to fly images with smell could also be compared to responses to fly images without smell. Images of male and female conspecifics with and without pheromones could be presented to female and male salticids, with potentially interesting results. Additionally, to explore the intriguing second peak phenomenon, it would be interesting to replicate experiments described in this thesis extending the number of trials to, for example, 200, to test if the response decrement trend looks the same, or if the second peak phenomenon occurs more than once.

Particularly promising avenues for further research include drug studies in salticids, which could be expanded by running another paired test with a drug that is not a CNS stimulant to see if that changes the response decrement. It would also be very interesting to test associative learning in salticids. For example, the prawn-in-a-tube procedure (Messenger, 1973) was used to study associative learning in cuttlefish. Purdy et al. (2006) measured the decline in attack responses in cuttlefish, *Sepia pharaonis*, when they were presented with a prawn in a clear tube. The authors found evidence that the response decrement was best viewed as a result of associative processes (i.e., the cuttlefish learned that they could not get the prawn, so stopped attacks). It would be helpful to replicate the prawn-in-a-tube procedure, in a modified form, in *T. planiceps.* On occasion, I observed that when salticids saw flies in the clear jar the flies were transported in for feeding, they would pounce onto the transporting jar in the precise location of a fly, presumably in an attempt to feed. Salticids would often try more than once, so it would be feasible to observe and measure the progression of jumping responses. For example, live flies could be placed in a clear closed container and presented to a salticid to see if jumping responses ceased after a certain number of attempts.

Conclusions

The finding that cognitive behaviour can potentially be facilitated in such a small brain holds enormous potential, especially when coupled with CNS stimulant drugs, as has been often used in honeybee studies. My results contribute to unravelling questions such as how salticids can process large amounts of information with their tiny brains and how they use this 'data' to make decisions. Further investigation of the processing power of salticid brains could have applications in micro-robotics. Given that the patterns observed in *T. planiceps* resembled those observed in humans,

these findings may also provide insight for human vigilance studies. Certainly, I hope my research encourages scientists to think about salticids in a new light.

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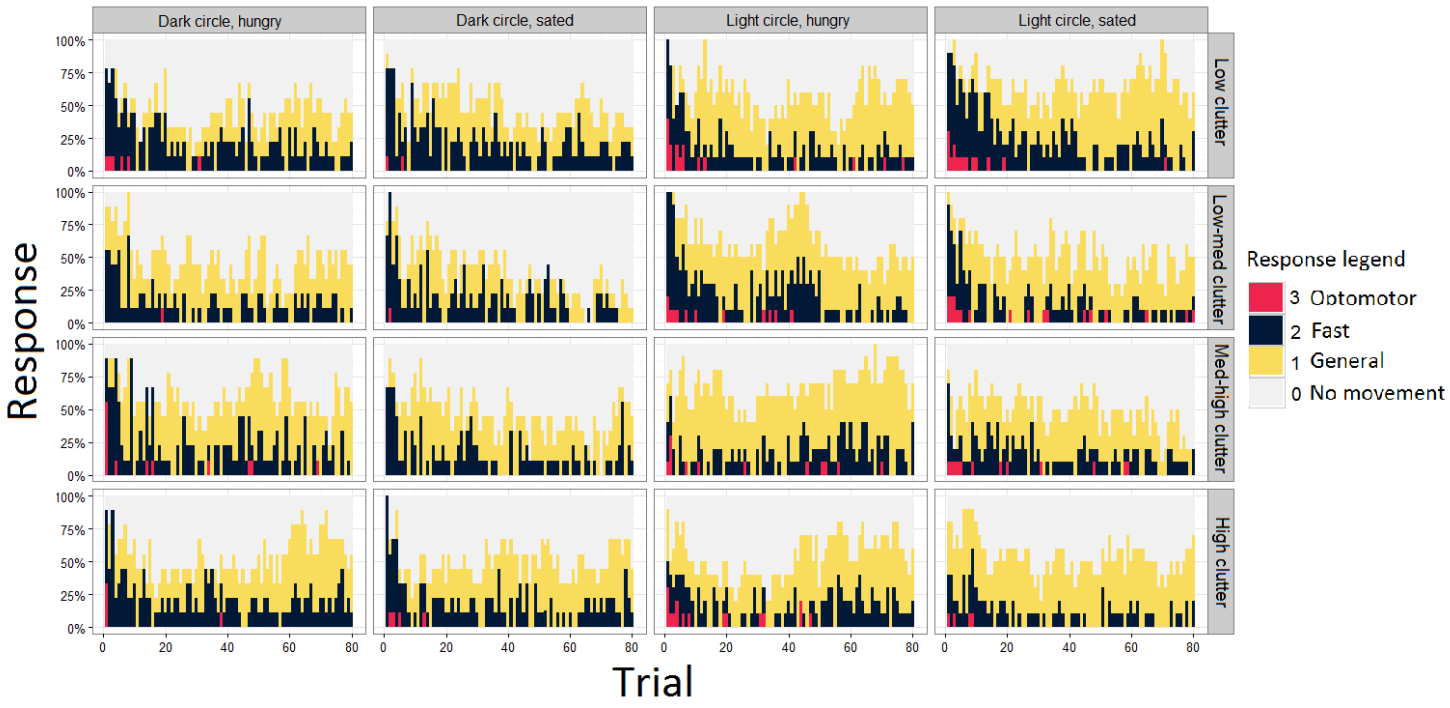
Appendices

Appendix 1

Chapter 2: vigilance in jumping spiders – supplementary information

Raw data: plotted in R© package ggplot2:



Model selection: potential model formulas compared in R©:

```
m <- clmm(Response ~ Trial + (1 + Trial | Spider)

mTD <- clmm(Response ~ (Trial + dot)^2 + (1 + Trial | Spider)

mTH <- clmm(Response ~ (Trial + Hunger)^2 + (1 + Trial | Spider)

mTC <- clmm(Response ~ (Trial + clutter)^2 + (1 + Trial | Spider)

mTDH <- clmm(Response ~ (Trial + dot + Hunger)^2 + (1 + Trial | Spider)

mTDC <- clmm(Response ~ (Trial + dot + clutter)^2 + (1 + Trial | Spider)

mTHC <- clmm(Response ~ (Trial + Hunger + clutter)^2 + (1 + Trial | Spider)

mTDHC <- clmm(Response ~ (Trial + dot + clutter + Hunger)^2 + (1 + Trial | Spider)

mTDHC3 <- clmm(Response ~ (Trial + dot + clutter + Hunger)^3 + (1 + Trial | Spider)

mTDHC4 <- clmm(Response ~ (Trial + dot + clutter + Hunger)^4 + (1 + Trial | Spider)
```

Model selection: R© package 'AICcmodavg' ranking models based on Akaike's information criterion

```
Model selection based on AICc:

        K     AICc Delta_AICc AICcWt Cum.Wt        LL
mTDHC4 37 24599.18       0.00      1      1 -12262.47
mTDHC  24 24623.66      24.48      0      1 -12287.78
mTDHC3 34 24624.17      24.99      0      1 -12277.99
mTHC   18 24666.01      66.83      0      1 -12314.98
mTDC   18 24764.16     164.98      0      1 -12364.05
mTC    13 24806.39     207.21      0      1 -12390.18
mTH     9 24837.13     237.95      0      1 -12409.56
mTDH   12 24839.63     240.45      0      1 -12407.80
m       7 24903.77     304.59      0      1 -12444.88
mTD     9 24905.15     305.97      0      1 -12443.57
```

Note: higher order interactions (mTDHC4 mTDHC3) were excluded from consideration because of prior knowledge of the nature of the data.

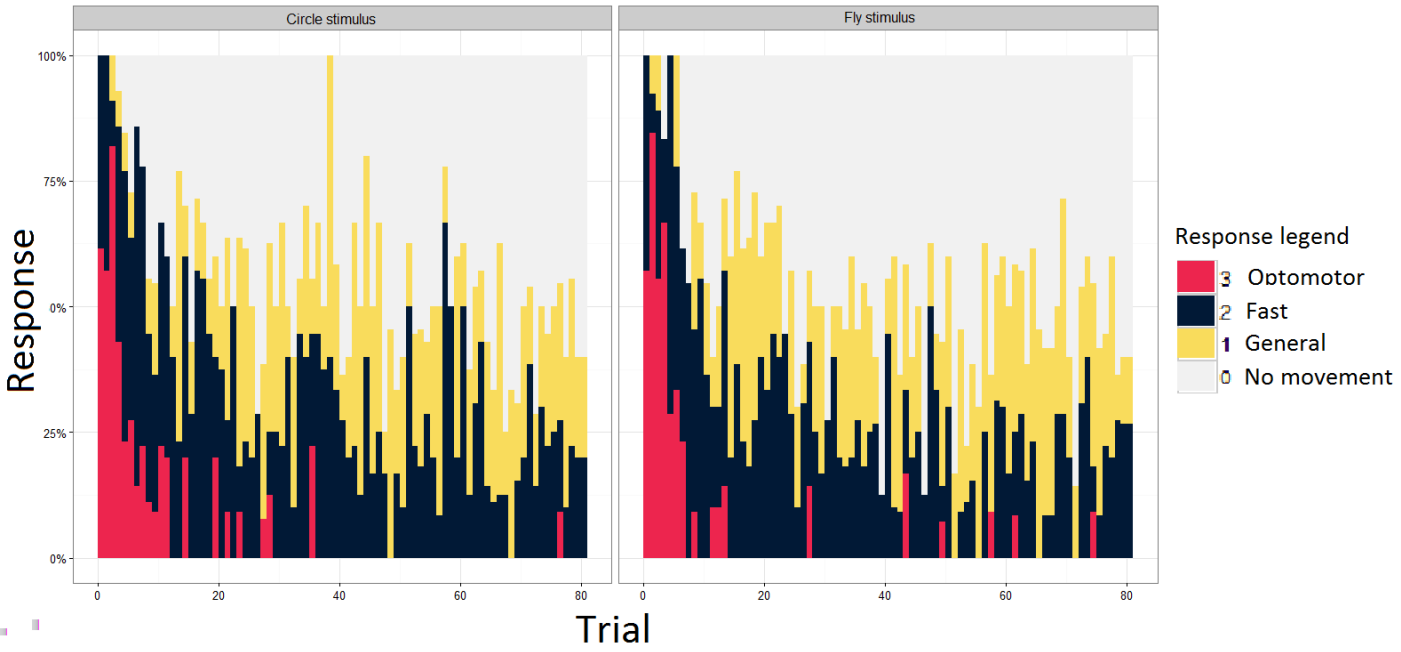
Coefficient output: raw coefficient data from the selected model (mTDHC)

```
Cumulative Link Mixed Model fitted with the Laplace approximation

formula: Response ~ (Trial + dot + clutter + Hunger)^2 + (1 + Trial | Spider)

 link  threshold nobs  logLik    AIC      niter       max.grad cond.H
 logit flexible  12159 -12287.78 24623.56 5591(25762) 1.51e+01 4.5e+05

Random effects:
 Groups Name        Variance  Std.Dev. Corr
 Spider (Intercept) 4.138e-01 0.643277
        Trial       5.077e-05 0.007125 -0.396
Number of groups:  Spider 19

Coefficients:
                  Estimate Std. Error z value Pr(>|z|)
Trial             0.012954   0.003087   4.196 2.71e-05 ***
dotL             -0.231559   0.313021  -0.740 0.459448
clutter2         -0.299034   0.126183  -2.370 0.017796 *
clutter3         -0.286854   0.125197  -2.291 0.021951 *
clutter4          0.169784   0.126794   1.339 0.180553
HungerS          -0.665438   0.102539  -6.490 8.61e-11 ***
Trial:dotL       -0.005177   0.003640  -1.422 0.154919
Trial:clutter2    0.007578   0.002233   3.393 0.000691 ***
Trial:clutter3   -0.005522   0.002181  -2.532 0.011354 *
Trial:clutter4   -0.012315   0.002187  -5.631 1.79e-08 ***
Trial:HungerS     0.010350   0.001555   6.654 2.85e-11 ***
dotL:clutter2    -0.128857   0.101959  -1.264 0.206294
dotL:clutter3     0.311887   0.099895   3.122 0.001795 **
dotL:clutter4     0.516969   0.099990   5.170 2.34e-07 ***
dotL:HungerS     -0.069053   0.071089  -0.971 0.331365
clutter2:HungerS  0.650117   0.101069   6.432 1.26e-10 ***
clutter3:HungerS  0.842097   0.099452   8.467  < 2e-16 ***
clutter4:HungerS  0.433002   0.099293   4.361 1.30e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Threshold coefficients:
    Estimate Std. Error z value
3|2  -4.4620     0.2525 -17.671
2|1  -1.3922     0.2390  -5.826
1|0   0.3945     0.2386   1.653
```

Appendix 2

Chapter 3: Biological relevance – supplementary information

Raw data: plotted in R© package ggplot2:



Stimuli used:

Model selection: Potential model formulas to test for the significance of stimulus side presentation and stimulus type. Bait = stimulus used (fly or circle)

```
m <- clmm(resp ~ Trial + (1 + Trial | Spider), data=dat)
n <- clmm(resp ~ (Trial + Bait)^2 + (1 + Trial | Spider), data=dat)
n1 <- clmm(resp ~ (Trial + Bait + Stimulus)^2 + (1 + Trial | Spider), data=dat)
n2 <- clmm(resp ~ (Trial + Stimulus)^2 + (1 + Trial | Spider), data=dat)\
```

Model selection: R© package 'AICcmodavg' ranking models based on Akaike's information

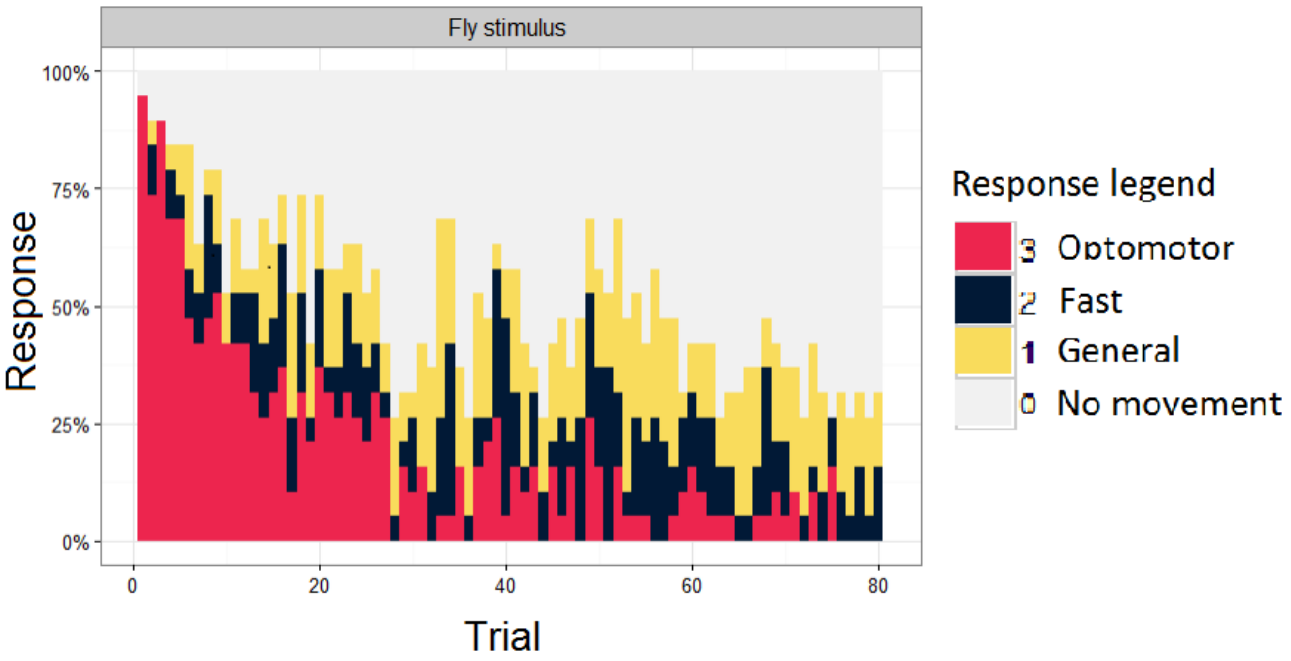
```
Model selection based on AICc:
    K    AICc Delta_AICc AICcWt Cum.Wt       LL
m   7 3344.36       0.00   0.48   0.48 -1665.14
n   9 3344.60       0.24   0.42   0.90 -1663.24
n2  9 3348.06       3.71   0.07   0.97 -1664.98
n1 12 3350.03       5.67   0.03   1.00 -1662.91
```

Coefficient output: raw coefficient data from the selected model

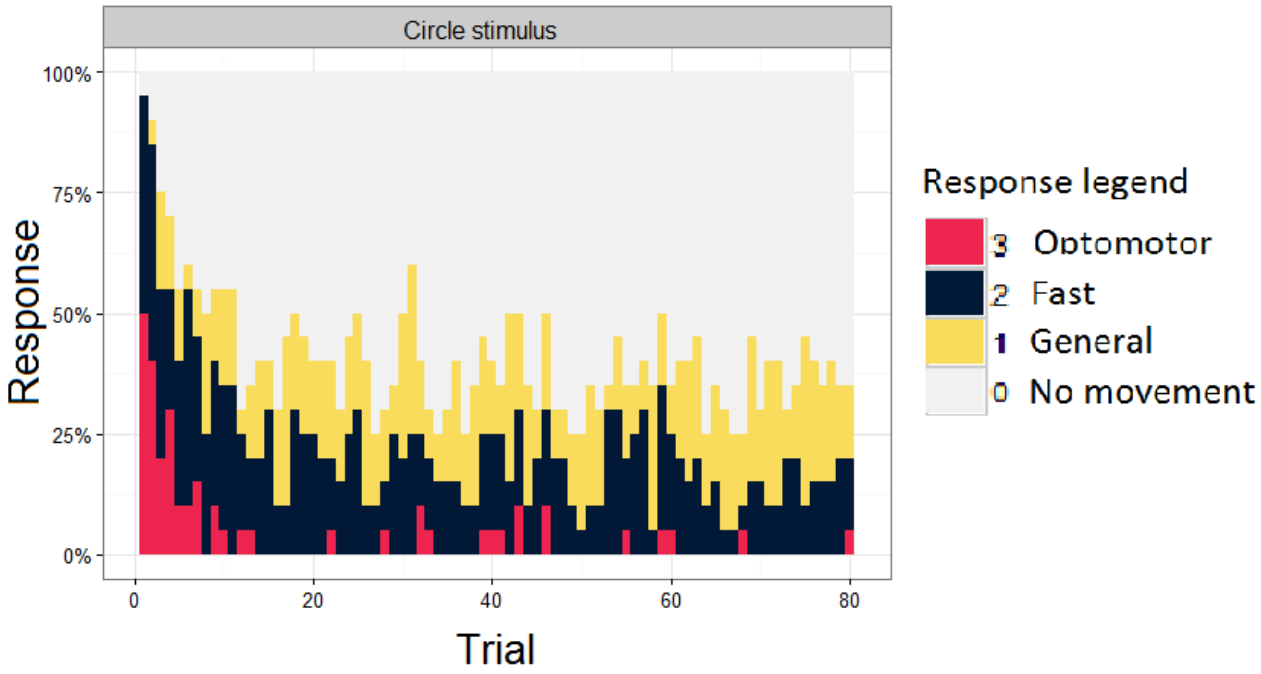
```
Cumulative Link Mixed Model fitted with the Laplace approximation

formula: resp ~ Trial + (1 + Trial | Spider)
data:    dat

 link  threshold nobs logLik   AIC     niter     max.grad cond.H
 logit flexible  1600 -1665.14 3344.28 640(3246) 8.37e-03 1.3e+04

Random effects:
 Groups Name        Variance Std.Dev. Corr
 Spider (Intercept) 1.756880 1.32547
        Trial       0.000991 0.03148  -0.585
Number of groups:  Spider 20

Coefficients:
      Estimate Std. Error z value Pr(>|z|)
Trial 0.037150   0.007594   4.892 9.99e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Threshold coefficients:
    Estimate Std. Error z value
3|2  -2.1101     0.3274  -6.445
2|1   0.3462     0.3174   1.091
1|0   1.6369     0.3193   5.126
```

Independent tests

Fly experiment:



Circle experiment:



Model selection: Potential model formulas to test for the significance of stimulus type. Bait = stimulus used (fly or circle). Data were from two different experiments merged into one file.

```
m <- clmm(Response ~ Trial + (1 + Trial | Spider), data=dat)

mr <- clmm(Response ~ (Trial + Bait)^2 + (1 + Trial | Spider), data=dat)
```

Model selection: R© package 'AICcmodavg' ranking models based on Akaike's information

```
Model selection based on AICc:

   K    AICc Delta_AICc AICcWt Cum.Wt       LL
mr 9 6449.13       0.00      1      1 -3215.54
m  7 6467.58      18.44      0      1 -3226.77
```

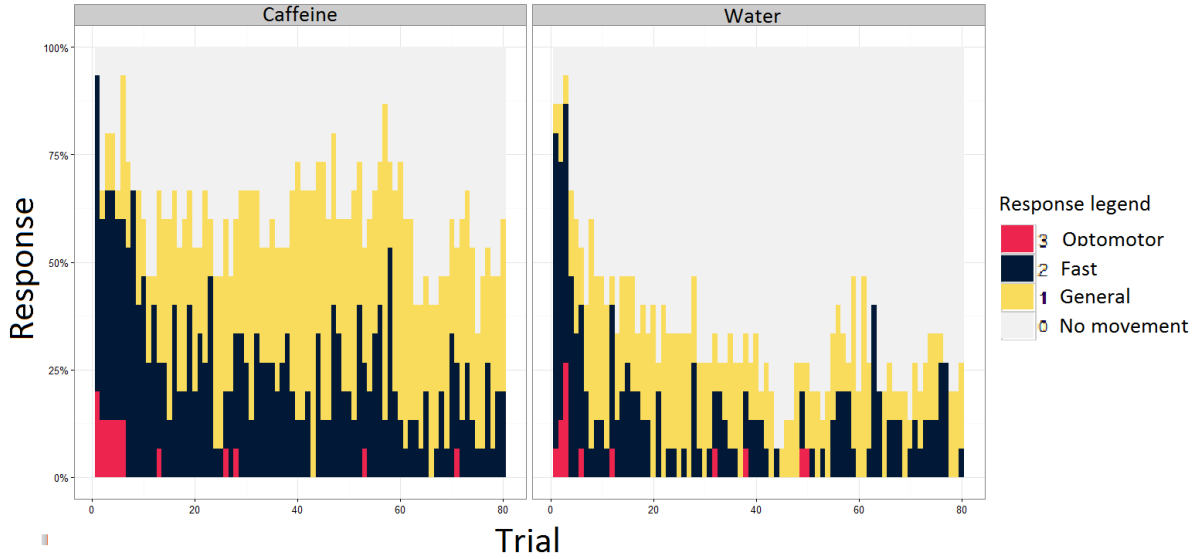
Coefficient output: raw coefficient data from the selected model

```
Cumulative Link Mixed Model fitted with the Laplace approximation

formula: Response ~ (Trial + Bait)^2 + (1 + Trial | Spider)
data:    dat

 link  threshold nobs logLik   AIC     niter     max.grad cond.H
 logit flexible  3120 -3215.54 6449.07 986(5036) 2.35e-02 2.1e+04

Random effects:
 Groups Name        Variance  Std.Dev. Corr
 Spider (Intercept) 0.6907370 0.83111
        Trial       0.0004188 0.02047  -0.045
Number of groups:  Spider 35

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
Trial          0.024225   0.005342   4.535 5.76e-06 ***
BaitFly       -1.662694   0.324322  -5.127 2.95e-07 ***
Trial:BaitFly  0.024863   0.008026   3.098  0.00195 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Threshold coefficients:
    Estimate Std. Error z value
3|2  -2.0577     0.2199  -9.357
2|1  -0.6926     0.2151  -3.220
1|0   0.3196     0.2148   1.488
```

Appendix 3

Chapter 4: The effect of caffeine on the response decrement in *T. planiceps*

Raw data: plotted in R© package ggplot2:



Model selection: potential model formulas compared in R©:

```
Caf <- clmm(Response ~ (Caffeine + Trial)^2 + (1 + Trial | Spider), data=dat)

noC <- clmm(Response ~ Trial + (1 + Trial | Spider), data=dat)
```

Model selection: R© package 'AICcmodavg' ranking models based on Akaike's information criterion

```
Model selection based on AICc:

    K    AICc Delta_AICc AICcWt Cum.Wt       LL
Caf 9 4328.12       0.00      1      1 -2155.02
noC 7 4553.56     225.43      0      1 -2269.75
```

Coefficient output: raw coefficient data from the selected model (Caf)

```
Cumulative Link Mixed Model fitted with the Laplace approximation

formula: Response ~ (Caffeine + Trial)^2 + (1 + Trial | Spider) data: dat

 link  threshold nobs logLik   AIC     niter      max.grad cond.H
 logit flexible  2400 -2155.02 4328.05 1176(5882) 6.54e-02 1.0e+05

Random effects:
 Groups Name        Variance  Std.Dev. Corr
 Spider (Intercept) 1.7004109 1.30400
        Trial       0.0001948 0.01396  -0.573
Number of groups:  Spider 15

Coefficients:
               Estimate Std. Error z value Pr(>|z|)
CaffeineW      0.966027   0.175406   5.507 3.64e-08 ***
Trial          0.018320   0.004444   4.122 3.75e-05 ***
CaffeineW:Trial 0.008659   0.003958   2.188   0.0287 *
 ---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Threshold coefficients:
    Estimate Std. Error z value
3|2  -3.7564     0.3950  -9.510
2|1  -0.4690     0.3578  -1.311
1|0   1.1519     0.3587   3.211
```

Coefficient output: raw coefficient data from the selected model (Caf) , rearranging the order of coefficients to get out of the intercept.

```
Cumulative Link Mixed Model fitted with the Laplace approximation

formula: Response ~ (Caffeine + Trial)^2 + (1 + Trial | Spider)
data:    dat

 link  threshold nobs logLik   AIC     niter      max.grad cond.H
 logit flexible  2400 -2155.02 4328.05 1149(5747) 6.91e-02 1.3e+05

Random effects:
 Groups Name        Variance  Std.Dev. Corr
 Spider (Intercept) 1.7004199 1.30400
        Trial       0.0001948 0.01396  -0.573
Number of groups:  Spider 15

Coefficients:
               Estimate Std. Error z value Pr(>|z|)
CaffeineC      -0.966028   0.175407  -5.507 3.64e-08 ***
Trial           0.026979   0.004831   5.584 2.34e-08 ***
CaffeineC:Trial -0.008659   0.003958  -2.188   0.0287 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Threshold coefficients:
    Estimate Std. Error z value
3|2  -4.7224     0.4068 -11.608
2|1  -1.4351     0.3650  -3.932
1|0   0.1858     0.3633   0.512
```